US008470586B2

(12) **United States Patent**
Wu et al.

(10) **Patent No.: US 8,470,586 B2**
(45) **Date of Patent: Jun. 25, 2013**

(54) **PROCESSING POLYNUCLEOTIDE-CONTAINING SAMPLES**

(75) Inventors: **Betty Wu**, Canton, MI (US); **John S. Althaus**, Saline, MI (US); **Sundaresh N. Brahmasandra**, Ann Arbor, MI (US); **Kalyan Handique**, Ypsilanti, MI (US); **Nikhil Phadke**, Ann Arbor, MI (US)

(73) Assignee: **Handylab, Inc.**, Franklin Lakes, NJ (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 711 days.

(21) Appl. No.: **11/579,353**

(22) PCT Filed: **May 3, 2005**

(86) PCT No.: **PCT/US2005/015345**

§ 371 (c)(1),
(2), (4) Date: **Jan. 10, 2008**

(87) PCT Pub. No.: **WO2005/018620**

PCT Pub. Date: **Nov. 17, 2005**

(65) **Prior Publication Data**

US 2008/0262213 A1 Oct. 23, 2008

Related U.S. Application Data

(60) Provisional application No. 60/645,784, filed on Jan. 21, 2005, provisional application No. 60/567,174, filed on May 3, 2004.

(51) **Int. Cl.**

C12M 1/00	(2006.01)
B01D 21/00	(2006.01)
B01L 99/00	(2010.01)

(52) **U.S. Cl.**
USPC **435/283.1**; 435/288.4; 435/288.5; 435/288.6; 422/68.1; 422/502; 422/527; 422/537

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

1,434,314 A 10/1922 Raich
1,616,419 A 2/1927 Wilson

(Continued)

FOREIGN PATENT DOCUMENTS

CA 2294819 1/1999
EP 0766256 4/1997

(Continued)

OTHER PUBLICATIONS

International Search Report, dated Oct. 6, 2008, issued in International Application No. PCT/US2008/69895.

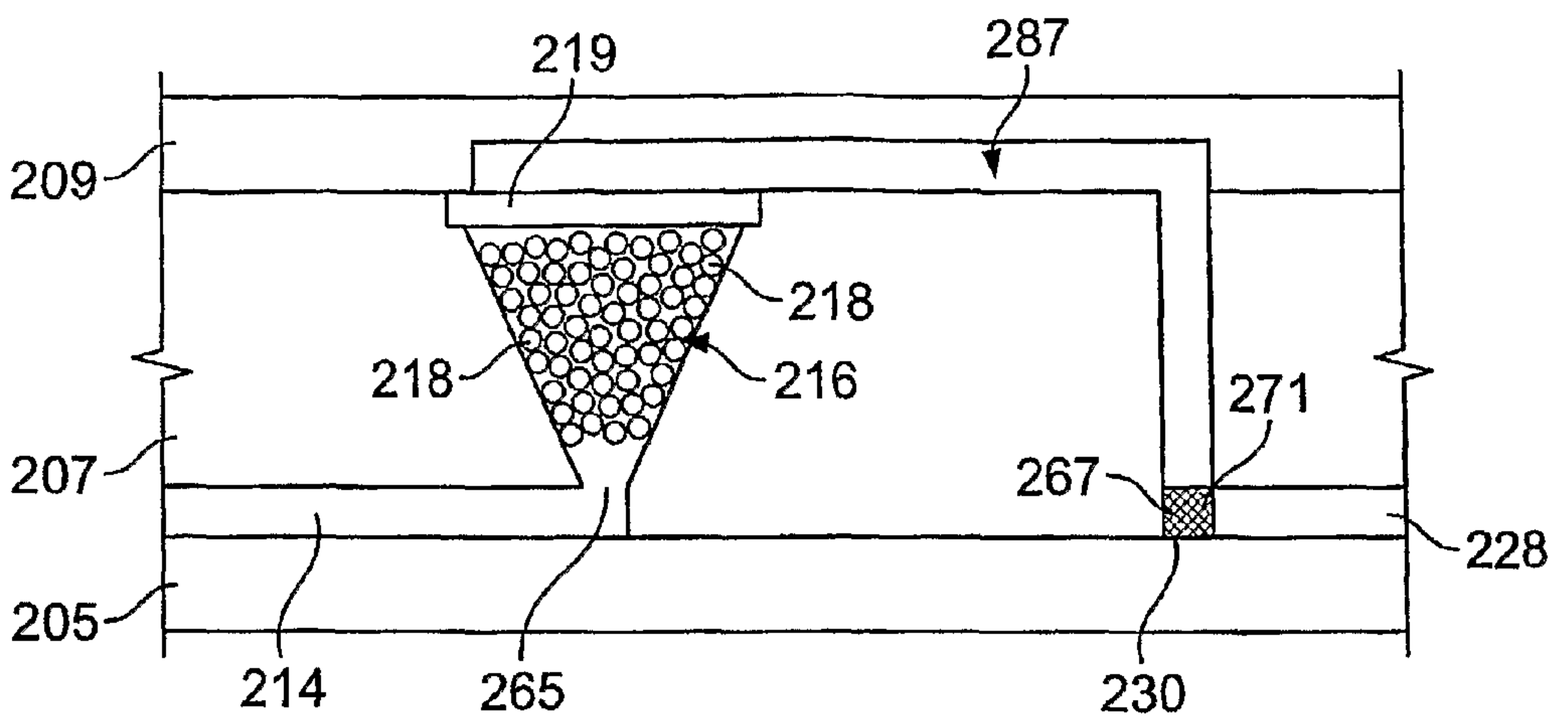
(Continued)

Primary Examiner — Betty Forman
(74) *Attorney, Agent, or Firm* — Knobbe Martens Olson & Bear LLP

(57) **ABSTRACT**

Methods and systems for processing polynucleotides (e.g., DNA) are disclosed. A processing region includes one or more surfaces (e.g., particle surfaces) modified with ligands that regain polynucleotides under a first set of conditions (e.g., temperature and pH) and release the polynucleotides under a second set of conditions (e.g., higher temperature and/or more basic pH). The processing region can be used to, for example, concentrate polynucleotides of a sample and/or separate inhibitors of amplification reactions from the polynucleotides. Microfluidic devices with a processing region are disclosed.

28 Claims, 19 Drawing Sheets



U.S. PATENT DOCUMENTS

1,733,401	A		8/1930	Lovekin
3,528,449	A		9/1970	Witte et al.
3,813,316	A		5/1974	Chakrabarty et al.
3,985,649	A		10/1976	Eddelman
4,018,089	A		4/1977	Dzula et al.
4,018,652	A		4/1977	Lanham et al.
4,038,192	A		7/1977	Serur
4,055,395	A		10/1977	Honkawa et al.
D249,706	S		9/1978	Adamski
4,139,005	A		2/1979	Dickey
D252,157	S		6/1979	Kronish et al.
D252,341	S		7/1979	Thomas
D254,687	S		4/1980	Fadler et al.
4,212,744	A		7/1980	Oota
D261,033	S		9/1981	Armbruster
D261,173	S		10/1981	Armbruster
4,301,412	A		11/1981	Hill et al.
4,439,526	A		3/1984	Columbus
4,457,329	A		7/1984	Werley et al.
4,466,740	A		8/1984	Kano et al.
4,504,582	A		3/1985	Swann
4,522,786	A		6/1985	Ebersole
D279,817	S		7/1985	Chen et al.
4,599,315	A		7/1986	Terasaki et al.
4,612,873	A		9/1986	Eberle
4,612,959	A		9/1986	Costello
D288,478	S		2/1987	Carlson et al.
4,654,127	A		3/1987	Baker et al.
4,673,657	A		6/1987	Christian
4,683,195	A		7/1987	Mullis et al.
4,683,202	A		7/1987	Mullis
D292,735	S		11/1987	Lovborg
4,720,374	A		1/1988	Ramachandran
4,798,693	A		1/1989	Mase et al.
4,800,022	A		1/1989	Leonard
4,841,786	A		6/1989	Schulz
D302,294	S		7/1989	Hillman
4,871,779	A		10/1989	Killat et al.
4,895,650	A		1/1990	Wang
4,919,829	A		4/1990	Gates et al.
4,921,809	A		5/1990	Shiff et al.
4,935,342	A		6/1990	Seligson et al.
4,946,562	A		8/1990	Guruswamy
4,949,742	A		8/1990	Rando et al.
D310,413	S		9/1990	Bigler et al.
4,963,498	A		10/1990	Hillman et al.
4,967,950	A		11/1990	Legg et al.
4,978,502	A		12/1990	Dole et al.
4,978,622	A		12/1990	Mishell et al.
4,989,626	A		2/1991	Takagi et al.
5,001,417	A		3/1991	Pumphrey et al.
5,004,583	A		4/1991	Guruswamy et al.
5,048,554	A		9/1991	Kremer
5,053,199	A		10/1991	Keiser et al.
5,060,823	A	*	10/1991	Perlman 222/1
5,061,336	A		10/1991	Soane
5,064,618	A		11/1991	Baker et al.
5,071,531	A		12/1991	Soane
5,091,328	A		2/1992	Miller
D324,426	S		3/1992	Fan et al.
5,096,669	A		3/1992	Lauks et al.
5,126,002	A		6/1992	Iwata et al.
5,126,022	A		6/1992	Soane et al.
D328,135	S		7/1992	Fan et al.
D328,794	S		8/1992	Frenkel et al.
5,135,627	A		8/1992	Soane
5,135,872	A		8/1992	Pouletty et al.
5,147,606	A		9/1992	Charlton et al.
5,169,512	A		12/1992	Wiedenmann et al.
D333,522	S		2/1993	Gianino
5,186,339	A		2/1993	Heissler
5,192,507	A		3/1993	Taylor et al.
5,208,163	A		5/1993	Charlton et al.
5,223,226	A		6/1993	Whittmer et al.
D338,275	S		8/1993	Fischer et al.
5,250,263	A		10/1993	Manz
5,252,743	A		10/1993	Barrett et al.
5,256,376	A		10/1993	Callan et al.
5,275,787	A		1/1994	Yuguchi et al.
5,282,950	A		2/1994	Dietze et al.
5,296,375	A		3/1994	Kricka et al.
5,304,477	A		4/1994	Nagoh et al.
5,304,487	A		4/1994	Wilding et al.
D347,478	S		5/1994	Pinkney
5,311,896	A		5/1994	Kaartinen
5,311,996	A		5/1994	Duffy et al.
5,316,727	A		5/1994	Suzuki et al.
5,327,038	A		7/1994	Culp
5,339,486	A		8/1994	Persic, Jr.
D351,475	S		10/1994	Gerber
D351,913	S		10/1994	Hieb et al.
5,364,591	A		11/1994	Green et al.
5,372,946	A		12/1994	Cusak et al.
5,374,395	A		12/1994	Robinson
5,389,339	A		2/1995	Petschek et al.
5,397,709	A		3/1995	Berndt
5,401,465	A		3/1995	Smethers et al.
5,411,708	A		5/1995	Moscetta
5,414,245	A		5/1995	Hackleman
5,416,000	A		5/1995	Allen et al.
5,422,271	A		6/1995	Chen et al.
5,422,284	A		6/1995	Lau
5,427,946	A		6/1995	Kricka et al.
5,474,796	A		12/1995	Brennan
D366,116	S		1/1996	Biskupski
5,486,335	A		1/1996	Wilding et al.
5,494,639	A		2/1996	Grzegorzewski
5,498,392	A		3/1996	Wilding et al.
5,503,803	A		4/1996	Brown
5,516,410	A		5/1996	Schneider et al.
5,519,635	A		5/1996	Miyake et al.
5,529,677	A		6/1996	Schneider et al.
5,559,432	A		9/1996	Logue
5,565,171	A		10/1996	Dovichi et al.
5,569,364	A		10/1996	Hooper et al.
5,578,270	A		11/1996	Reichler et al.
5,578,818	A		11/1996	Kain et al.
5,579,928	A		12/1996	Anukwuem
5,580,523	A		12/1996	Bard
5,582,884	A		12/1996	Ball et al.
5,585,069	A		12/1996	Zanzucchi et al.
5,585,089	A		12/1996	Queen et al.
5,585,242	A		12/1996	Bouma et al.
5,587,128	A		12/1996	Wilding et al.
5,589,136	A		12/1996	Northrup et al.
5,593,838	A		1/1997	Zanzucchi et al.
5,595,708	A		1/1997	Berndt
5,599,432	A		2/1997	Manz et al.
5,599,503	A		2/1997	Manz et al.
5,599,667	A		2/1997	Arnold, Jr. et al.
5,601,727	A		2/1997	Bormann et al.
5,603,351	A		2/1997	Cherukuri et al.
5,605,662	A		2/1997	Heller et al.
D378,782	S		4/1997	LaBarbera et al.
5,628,890	A		5/1997	Carter et al.
5,630,920	A		5/1997	Friese et al.
5,631,337	A		5/1997	Sassi et al.
5,632,876	A		5/1997	Zanzucchi et al.
5,632,957	A		5/1997	Heller et al.
5,635,358	A		6/1997	Wilding et al.
5,637,469	A		6/1997	Wilding et al.
5,639,423	A		6/1997	Northrup et al.
5,643,738	A		7/1997	Zanzucchi et al.
5,646,039	A		7/1997	Northrup et al.
5,647,994	A		7/1997	Tuunanen et al.
5,651,839	A		7/1997	Rauf
5,652,149	A		7/1997	Mileaf et al.
D382,346	S		8/1997	Buhler et al.
D382,647	S		8/1997	Staples et al.
5,667,976	A		9/1997	Van Ness et al.
5,671,303	A		9/1997	Shieh et al.
5,674,394	A		10/1997	Whitmore
5,674,742	A		10/1997	Northrup et al.
5,681,484	A		10/1997	Zanzucchi et al.
5,681,529	A		10/1997	Taguchi et al.
5,683,657	A		11/1997	Mian
5,699,157	A		12/1997	Parce

5,700,637	A	12/1997	Southern
5,705,813	A	1/1998	Apffel et al.
5,726,026	A	3/1998	Wilding et al.
5,726,404	A	3/1998	Brody
5,726,944	A	3/1998	Taft et al.
5,731,212	A	3/1998	Gavin et al.
5,744,366	A	4/1998	Kricka et al.
5,747,666	A	5/1998	Willis
5,750,015	A	5/1998	Soane et al.
5,755,942	A	5/1998	Zanzucchi et al.
5,763,262	A	6/1998	Wong et al.
5,770,029	A	6/1998	Nelson et al.
5,770,388	A	6/1998	Vorpahl
5,772,966	A	6/1998	Maracas et al.
5,779,868	A	7/1998	Parce et al.
5,787,032	A	7/1998	Heller et al.
5,788,814	A	8/1998	Sun et al.
5,800,600	A	9/1998	Lima-Marques et al.
5,800,690	A	9/1998	Chow et al.
5,804,436	A	9/1998	Okun et al.
D399,959	S	10/1998	Prokop et al.
5,827,481	A	10/1998	Bente et al.
5,842,106	A	11/1998	Thaler et al.
5,842,787	A	12/1998	Kopf-Sill et al.
5,846,396	A	12/1998	Zanzucchi et al.
5,849,208	A	12/1998	Hayes et al.
5,849,486	A	12/1998	Heller et al.
5,849,489	A	12/1998	Heller
5,849,598	A	12/1998	Wilson et al.
5,852,495	A	12/1998	Parce
5,856,174	A	1/1999	Lipshutz et al.
5,858,187	A	1/1999	Ramsey et al.
5,858,188	A	1/1999	Soane et al.
5,863,502	A	1/1999	Southgate et al.
5,863,708	A	1/1999	Zanzucchi et al.
5,863,801	A	1/1999	Southgate et al.
5,866,345	A	2/1999	Wilding et al.
5,869,004	A	2/1999	Parce et al.
5,869,244	A	2/1999	Martin et al.
5,872,010	A	2/1999	Karger et al.
5,872,623	A	2/1999	Stabile et al.
5,874,046	A	2/1999	Megerle
5,876,675	A	3/1999	Kennedy
5,880,071	A	3/1999	Parce et al.
5,882,465	A	3/1999	McReynolds
5,883,211	A	3/1999	Sassi et al.
5,885,432	A	3/1999	Hooper et al.
5,885,470	A	3/1999	Parce et al.
5,895,762	A	4/1999	Greenfield et al.
5,900,130	A	5/1999	Benvegnu et al.
5,912,124	A	6/1999	Kumar
5,912,134	A	6/1999	Shartle
5,916,522	A	6/1999	Boyd et al.
5,916,776	A	6/1999	Kumar
5,919,646	A	7/1999	Okun et al.
5,919,711	A	7/1999	Boyd et al.
5,922,591	A	7/1999	Anderson et al.
5,927,547	A	7/1999	Papen et al.
5,928,880	A	7/1999	Wilding et al.
5,929,208	A	7/1999	Heller et al.
D413,391	S	8/1999	Lapeus et al.
5,932,799	A	8/1999	Moles
5,935,401	A	8/1999	Amigo
5,939,291	A	8/1999	Loewy et al.
5,942,443	A	8/1999	Parce et al.
D413,677	S	9/1999	Dumitrescu et al.
5,948,227	A	9/1999	Dubrow
5,955,028	A	9/1999	Chow
5,955,029	A	9/1999	Wilding et al.
5,957,579	A	9/1999	Kopf-Sill et al.
5,958,203	A	9/1999	Parce et al.
5,958,694	A	9/1999	Nikiforov
5,959,221	A	9/1999	Boyd et al.
5,959,291	A	9/1999	Jensen
5,964,995	A	10/1999	Nikiforov et al.
5,964,997	A	10/1999	McBride
5,965,001	A	10/1999	Chow et al.
5,965,410	A	10/1999	Chow et al.
5,965,886	A	10/1999	Sauer et al.
5,968,745	A	10/1999	Thorp et al.
5,972,187	A	10/1999	Parce et al.
5,973,138	A	10/1999	Collis
D417,009	S	11/1999	Boyd
5,976,336	A	11/1999	Dubrow et al.
5,980,704	A	11/1999	Cherukuri et al.
5,980,719	A	11/1999	Cherukuri et al.
5,981,735	A	11/1999	Thatcher et al.
5,989,402	A	11/1999	Chow et al.
5,992,820	A	11/1999	Fare et al.
5,993,611	A	11/1999	Moroney, III et al.
5,993,750	A	11/1999	Ghosh et al.
5,997,708	A	12/1999	Craig
6,001,229	A	12/1999	Ramsey
6,001,231	A	12/1999	Kopf-Sill
6,001,307	A	12/1999	Naka et al.
6,004,515	A	12/1999	Parce et al.
6,007,690	A	12/1999	Nelson et al.
6,010,607	A	1/2000	Ramsey
6,010,608	A	1/2000	Ramsey
6,010,627	A	1/2000	Hood, III
6,012,902	A	1/2000	Parce
D420,747	S	2/2000	Dumitrescu et al.
D421,130	S	2/2000	Cohen et al.
6,024,920	A	2/2000	Cunanan
D421,653	S	3/2000	Purcell
6,033,546	A	3/2000	Ramsey
6,043,080	A	3/2000	Lipshutz et al.
6,046,056	A	4/2000	Parce et al.
6,048,734	A	4/2000	Burns et al.
6,054,034	A	4/2000	Soane et al.
6,054,277	A	4/2000	Furcht et al.
6,056,860	A	5/2000	Amigo et al.
6,057,149	A	5/2000	Burns et al.
6,062,261	A	5/2000	Jacobson et al.
6,063,341	A	5/2000	Fassbind et al.
6,063,589	A	5/2000	Kellogg et al.
6,068,752	A	5/2000	Dubrow et al.
6,071,478	A	6/2000	Chow
6,074,725	A	6/2000	Kennedy
6,074,827	A	6/2000	Nelson et al.
D428,497	S	7/2000	Lapeus et al.
6,086,740	A	7/2000	Kennedy
6,096,509	A	8/2000	Okun et al.
6,100,541	A	8/2000	Nagle et al.
6,102,897	A	8/2000	Lang
6,103,537	A	8/2000	Ullman et al.
6,106,685	A	8/2000	McBride et al.
6,110,343	A	8/2000	Ramsey et al.
6,123,205	A	9/2000	Dumitrescu et al.
6,123,798	A	9/2000	Gandhi et al.
6,130,098	A	10/2000	Handique et al.
6,132,580	A	10/2000	Mathies et al.
6,132,684	A	10/2000	Marino
6,133,436	A	10/2000	Koster et al.
D433,759	S	11/2000	Mathis et al.
6,143,250	A	11/2000	Tajima
6,149,787	A	11/2000	Chow et al.
6,156,199	A	12/2000	Zuk, Jr.
6,158,269	A	12/2000	Dorenkott et al.
6,167,910	B1	1/2001	Chow
6,168,948	B1	1/2001	Anderson et al.
6,171,850	B1	1/2001	Nagle et al.
6,174,675	B1	1/2001	Chow et al.
6,180,950	B1	1/2001	Olsen
D438,311	S	2/2001	Yamanishi et al.
D438,632	S	3/2001	Miller
D438,633	S	3/2001	Miller
6,197,595	B1	3/2001	Anderson et al.
6,211,989	B1	4/2001	Wulf et al.
6,213,151	B1	4/2001	Jacobson et al.
6,221,600	B1	4/2001	MacLeod et al.
6,228,635	B1	5/2001	Armstrong et al.
6,232,072	B1	5/2001	Fisher
6,235,175	B1	5/2001	Dubrow et al.
6,235,313	B1	5/2001	Mathiowitz et al.
6,235,471	B1	5/2001	Knapp et al.
6,236,456	B1	5/2001	Giebeler et al.
6,236,581	B1	5/2001	Lines et al.

6,251,343	B1	6/2001	Dubrow et al.
6,254,826	B1	7/2001	Acosta et al.
6,259,635	B1	7/2001	Torelli et al.
6,261,431	B1	7/2001	Mathies et al.
6,267,858	B1	7/2001	Parce et al.
D446,306	S	8/2001	Ochi et al.
6,271,021	B1	8/2001	Burns et al.
6,274,089	B1	8/2001	Chow et al.
6,280,967	B1	8/2001	Ransom et al.
6,281,008	B1	8/2001	Komai et al.
6,284,113	B1	9/2001	Bjornson et al.
6,287,254	B1	9/2001	Dodds
6,287,774	B1	9/2001	Nikiforov
6,291,248	B1	9/2001	Haj-Ahmad
6,294,063	B1	9/2001	Becker et al.
6,302,134	B1	10/2001	Kellogg et al.
6,302,304	B1	10/2001	Spencer
6,303,343	B1	10/2001	Kopf-Sill
6,306,273	B1	10/2001	Wainright et al.
6,306,590	B1	10/2001	Mehta et al.
6,319,469	B1	11/2001	Mian et al.
6,322,683	B1	11/2001	Wolk et al.
6,326,083	B1	12/2001	Yang et al.
6,326,211	B1	12/2001	Anderson et al.
6,334,980	B1	1/2002	Hayes et al.
6,337,435	B1	1/2002	Chu et al.
6,353,475	B1	3/2002	Jensen et al.
6,358,387	B1	3/2002	Kopf-sill et al.
6,366,924	B1	4/2002	Parce
6,368,871	B1	4/2002	Christel et al.
6,370,206	B1	4/2002	Schenk
6,375,185	B1	4/2002	Lin
6,375,901	B1	4/2002	Robotti et al.
6,379,884	B2	4/2002	Wada et al.
6,379,929	B1	4/2002	Burns et al.
6,379,974	B1	4/2002	Parce et al.
6,382,254	B1	5/2002	Yang et al.
6,391,541	B1	5/2002	Petersen et al.
6,391,623	B1	5/2002	Besemer et al.
6,395,161	B1	5/2002	Schneider et al.
6,398,956	B1	6/2002	Coville et al.
6,399,025	B1	6/2002	Chow
6,399,389	B1	6/2002	Parce et al.
6,399,952	B1	6/2002	Maher et al.
6,401,552	B1	6/2002	Elkins
6,403,338	B1	6/2002	Knapp et al.
6,408,878	B2	6/2002	Unger et al.
6,413,401	B1	7/2002	Chow et al.
6,416,642	B1	7/2002	Alajoki et al.
6,420,143	B1	7/2002	Kopf-sill
6,425,972	B1	7/2002	Mcreynolds
D461,906	S	8/2002	Pham
6,428,987	B2	8/2002	Franzen
6,430,512	B1	8/2002	Gallagher
6,432,366	B2	8/2002	Ruediger et al.
6,440,725	B1	8/2002	Pourahmadi et al.
D463,031	S	9/2002	Slomski et al.
6,444,461	B1	9/2002	Knapp et al.
6,447,661	B1	9/2002	Chow et al.
6,447,727	B1	9/2002	Parce et al.
6,448,064	B1	9/2002	Vo-Dinh et al.
6,453,928	B1	9/2002	Kaplan et al.
6,465,257	B1	10/2002	Parce et al.
6,468,761	B2	10/2002	Yang et al.
6,472,141	B2	10/2002	Nikiforov
6,475,364	B1	11/2002	Dubrow et al.
D467,348	S	12/2002	McMichael et al.
D467,349	S	12/2002	Niedbala et al.
6,488,897	B2	12/2002	Dubrow et al.
6,495,104	B1	12/2002	Unno et al.
6,498,497	B1	12/2002	Chow et al.
6,500,323	B1	12/2002	Chow et al.
6,500,390	B1	12/2002	Boulton et al.
D468,437	S	1/2003	McMenamy et al.
6,506,609	B1	1/2003	Wada et al.
6,509,193	B1	1/2003	Tajima
6,511,853	B1	1/2003	Kopf-sill et al.
D470,595	S	2/2003	Crisanti et al.
6,515,753	B2	2/2003	Maher et al.
6,517,783	B2	2/2003	Horner et al.
6,520,197	B2	2/2003	Deshmukh et al.
6,521,188	B1	2/2003	Webster
6,524,456	B1	2/2003	Ramsey et al.
6,524,790	B1	2/2003	Kopf-sill et al.
D472,324	S	3/2003	Rumore et al.
6,534,295	B2	3/2003	Tai et al.
6,537,771	B1	3/2003	Farinas et al.
6,540,896	B1	4/2003	Manz et al.
6,544,734	B1	4/2003	Briscoe et al.
6,547,942	B1	4/2003	Parce et al.
6,555,389	B1	4/2003	Ullman et al.
6,556,923	B2	4/2003	Gallagher et al.
D474,279	S	5/2003	Mayer et al.
D474,280	S	5/2003	Niedbala et al.
6,558,916	B2	5/2003	Veerapandian et al.
6,558,945	B1	5/2003	Kao
6,569,607	B2	5/2003	Mcreynolds
6,572,830	B1	6/2003	Burdon et al.
6,575,188	B2	6/2003	Parunak
6,576,459	B2	6/2003	Miles et al.
6,579,453	B1	6/2003	Bächler et al.
6,589,729	B2	7/2003	Chan et al.
6,592,821	B1	7/2003	Wada et al.
6,597,450	B1	7/2003	Andrews et al.
6,602,474	B1	8/2003	Tajima
6,613,211	B1	9/2003	Mccormick et al.
6,613,512	B1	9/2003	Kopf-sill et al.
6,613,580	B1	9/2003	Chow et al.
6,613,581	B1	9/2003	Wada et al.
6,614,030	B2	9/2003	Maher et al.
6,620,625	B2	9/2003	Wolk et al.
6,623,860	B2	9/2003	Hu et al.
6,627,406	B1	9/2003	Singh et al.
D480,814	S	10/2003	Lafferty et al.
6,632,655	B1	10/2003	Mehta et al.
6,633,785	B1	10/2003	Kasahara et al.
D482,796	S	11/2003	Oyama et al.
6,649,358	B1	11/2003	Parce et al.
6,664,104	B2	12/2003	Pourahmadi et al.
6,669,831	B2	12/2003	Chow et al.
6,670,153	B2	12/2003	Stern
D484,989	S	1/2004	Gebrian
6,681,616	B2	1/2004	Spaid et al.
6,681,788	B2	1/2004	Parce et al.
6,685,813	B2	2/2004	Williams et al.
6,692,700	B2	2/2004	Handique
6,695,009	B2	2/2004	Chien et al.
6,706,519	B1	3/2004	Kellogg et al.
6,720,148	B1	4/2004	Nikiforov
6,730,206	B2	5/2004	Ricco et al.
6,733,645	B1	5/2004	Chow
6,734,401	B2	5/2004	Bedingham et al.
6,737,026	B1	5/2004	Bergh et al.
6,740,518	B1	5/2004	Duong et al.
D491,272	S	6/2004	Alden et al.
D491,273	S	6/2004	Biegler et al.
D491,276	S	6/2004	Langille
6,750,661	B2	6/2004	Brooks et al.
6,752,966	B1	6/2004	Chazan
6,756,019	B1	6/2004	Dubrow et al.
6,766,817	B2	7/2004	da Silva
6,773,567	B1	8/2004	Wolk
6,777,184	B2	8/2004	Nikiforov et al.
6,783,962	B1	8/2004	Olander et al.
D495,805	S	9/2004	Lea et al.
6,787,015	B2	9/2004	Lackritz et al.
6,787,016	B2	9/2004	Tan et al.
6,790,328	B2	9/2004	Jacobson et al.
6,790,330	B2	9/2004	Gascoyne et al.
6,811,668	B1	11/2004	Berndt et al.
6,818,113	B2	11/2004	Williams et al.
6,819,027	B2	11/2004	Saraf
6,824,663	B1	11/2004	Boone
D499,813	S	12/2004	Wu
D500,142	S	12/2004	Crisanti et al.
6,827,831	B1	12/2004	Chow et al.
6,827,906	B1	12/2004	Bjornson et al.
6,838,156	B1	1/2005	Neyer et al.

6,838,680	B2	1/2005	Maher et al.	7,351,377	B2	4/2008	Chazan et al.
6,852,287	B2	2/2005	Ganesan	7,374,949	B2	5/2008	Kuriger
6,858,185	B1	2/2005	Kopf-sill et al.	7,390,460	B2	6/2008	Osawa et al.
6,859,698	B2	2/2005	Schmeisser	7,419,784	B2	9/2008	Dubrow et al.
6,861,035	B2	3/2005	Pham et al.	7,422,669	B2	9/2008	Jacobson et al.
6,878,540	B2	4/2005	Pourahmadi et al.	7,440,684	B2	10/2008	Spaid et al.
6,878,755	B2	4/2005	Singh et al.	7,476,313	B2	1/2009	Siddiqi
6,884,628	B2	4/2005	Hubbell et al.	7,494,577	B2	2/2009	Williams et al.
6,887,693	B2	5/2005	McMillan et al.	7,494,770	B2	2/2009	Wilding et al.
6,893,879	B2	5/2005	Petersen et al.	7,514,046	B2	4/2009	Kechagia et al.
6,900,889	B2	5/2005	Bjornson et al.	7,521,186	B2	4/2009	Burd Mehta
6,905,583	B2	6/2005	Wainright et al.	7,553,671	B2	6/2009	Sinclair et al.
6,905,612	B2	6/2005	Dorian et al.	7,595,197	B2	9/2009	Brasseur
6,906,797	B1	6/2005	Kao et al.	7,635,588	B2	12/2009	King et al.
6,908,594	B1	6/2005	Schaevitz et al.	7,645,581	B2	1/2010	Knapp et al.
6,911,183	B1	6/2005	Handique et al.	7,670,559	B2	3/2010	Chien et al.
6,914,137	B2	7/2005	Baker	7,674,431	B2	3/2010	Ganesan
6,915,679	B2	7/2005	Chien et al.	7,723,123	B1	5/2010	Murphy et al.
6,918,404	B2	7/2005	Dias da Silva	7,744,817	B2	6/2010	Bui
D508,999	S	8/2005	Fanning et al.	7,867,776	B2	1/2011	Kennedy et al.
6,939,451	B2	9/2005	Zhao et al.	7,892,819	B2	2/2011	Wilding et al.
6,942,771	B1	9/2005	Kayyem	8,088,616	B2	1/2012	Handique
6,958,392	B2	10/2005	Fomovskaia et al.	8,105,783	B2	1/2012	Handique
D512,155	S	11/2005	Matsumoto	8,133,671	B2	3/2012	Williams et al.
6,964,747	B2	11/2005	Banerjee et al.	8,182,763	B2	5/2012	Duffy et al.
6,977,163	B1	12/2005	Mehta	8,287,820	B2	10/2012	Williams et al.
6,984,516	B2	1/2006	Briscoe et al.	8,323,584	B2	12/2012	Ganesan
D515,707	S	2/2006	Shinohara et al.	8,323,900	B2	12/2012	Handique et al.
D516,221	S	2/2006	Wohlstadter et al.	8,324,372	B2	12/2012	Brahmasandra et al.
7,001,853	B1	2/2006	Brown et al.	2001/0023848	A1	9/2001	Gjerde et al.
7,004,184	B2	2/2006	Handique et al.	2001/0038450	A1	11/2001	McCaffrey et al.
D517,554	S	3/2006	Yanagisawa et al.	2001/0046702	A1	11/2001	Schembri
7,010,391	B2	3/2006	Handique et al.	2001/0055765	A1	12/2001	O'Keefe et al.
7,023,007	B2	4/2006	Gallagher	2002/0001848	A1	1/2002	Bedingham et al.
7,024,281	B1	4/2006	Unno	2002/0008053	A1	1/2002	Hansen et al.
7,036,667	B2	5/2006	Greenstein et al.	2002/0009015	A1	1/2002	Laugharn, Jr. et al.
7,037,416	B2	5/2006	Parce et al.	2002/0015667	A1	2/2002	Chow
7,038,472	B1	5/2006	Chien	2002/0021983	A1	2/2002	Comte et al.
7,039,527	B2	5/2006	Tripathi et al.	2002/0037499	A1	3/2002	Quake et al.
7,040,144	B2	5/2006	Spaid et al.	2002/0039783	A1	4/2002	McMillan et al.
D523,153	S	6/2006	Akashi et al.	2002/0053399	A1	5/2002	Soane et al.
7,055,695	B2	6/2006	Greenstein et al.	2002/0054835	A1	5/2002	Robotti et al.
7,060,171	B1	6/2006	Nikiforov et al.	2002/0055167	A1	5/2002	Pourahmadi et al.
7,066,586	B2	6/2006	da Silva	2002/0058332	A1	5/2002	Quake et al.
7,069,952	B1	7/2006	Mcreynolds et al.	2002/0060156	A1	5/2002	Mathies et al.
7,099,778	B2	8/2006	Chien	2002/0068357	A1	6/2002	Mathies et al.
D528,215	S	9/2006	Malmsater	2002/0141903	A1	10/2002	Parunak et al.
7,101,467	B2	9/2006	Spaid	2002/0142471	A1	10/2002	Handique et al.
7,105,304	B1	9/2006	Nikiforov et al.	2002/0143297	A1	10/2002	Francavilla et al.
D531,321	S	10/2006	Godfrey et al.	2002/0143437	A1	10/2002	Handique et al.
7,118,910	B2	10/2006	Unger et al.	2002/0155477	A1	10/2002	Ito
7,138,032	B2	11/2006	Gandhi et al.	2002/0169518	A1	11/2002	Luoma et al.
D534,280	S	12/2006	Gomm et al.	2002/0187557	A1	12/2002	Hobbs et al.
7,148,043	B2	12/2006	Kordunsky et al.	2003/0019522	A1	1/2003	Parunak
7,150,814	B1	12/2006	Parce et al.	2003/0049833	A1	3/2003	Chen et al.
7,150,999	B1	12/2006	Shuck	2003/0064507	A1	4/2003	Gallagher et al.
D535,403	S	1/2007	Isozaki et al.	2003/0070677	A1	4/2003	Handique et al.
7,160,423	B2	1/2007	Chien et al.	2003/0073106	A1	4/2003	Johansen et al.
7,161,356	B1	1/2007	Chien	2003/0083686	A1	5/2003	Freeman et al.
7,169,277	B2	1/2007	Ausserer et al.	2003/0087300	A1	5/2003	Knapp et al.
7,169,618	B2	1/2007	Skould	2003/0127327	A1	7/2003	Kurnik
D537,951	S	3/2007	Okamoto et al.	2003/0136679	A1	7/2003	Bohn et al.
D538,436	S	3/2007	Patadia et al.	2003/0186295	A1 *	10/2003	Colin et al. 435/6
7,192,557	B2	3/2007	Wu et al.	2003/0199081	A1	10/2003	Wilding et al.
7,195,986	B1	3/2007	Bousse et al.	2003/0211517	A1	11/2003	Carulli et al.
7,208,125	B1	4/2007	Dong	2004/0014238	A1	1/2004	Krug et al.
7,235,406	B1	6/2007	Woudenberg et al.	2004/0029258	A1	2/2004	Heaney et al.
7,247,274	B1	7/2007	Chow	2004/0029260	A1	2/2004	Hansen et al.
D548,841	S	8/2007	Brownell et al.	2004/0037739	A1	2/2004	McNeely et al.
D549,827	S	8/2007	Maeno et al.	2004/0053290	A1	3/2004	Terbrueggen et al.
7,252,928	B1	8/2007	Hafeman et al.	2004/0063217	A1	4/2004	Webster et al.
7,270,786	B2	9/2007	Parunak et al.	2004/0072278	A1	4/2004	Chou et al.
D554,069	S	10/2007	Bolotin et al.	2004/0072375	A1	4/2004	Gjerde et al.
D554,070	S	10/2007	Bolotin et al.	2004/0086956	A1	5/2004	Bachur, Jr.
7,276,330	B2	10/2007	Chow et al.	2004/0141887	A1	7/2004	Mainquist et al.
7,303,727	B1	12/2007	Dubrow et al.	2004/0151629	A1	8/2004	Pease et al.
7,323,140	B2	1/2008	Handique et al.	2004/0157220	A1	8/2004	Kurnool et al.
7,332,130	B2	2/2008	Handique	2004/0161788	A1 *	8/2004	Chen et al. 435/6
7,338,760	B2	3/2008	Gong et al.	2004/0189311	A1	9/2004	Glezer et al.

2004/0209331	A1	10/2004	Ririe
2004/0209354	A1	10/2004	Mathies et al.
2004/0219070	A1	11/2004	Handique
2004/0240097	A1	12/2004	Evans
2005/0009174	A1	1/2005	Nikiforov et al.
2005/0041525	A1	2/2005	Pugia et al.
2005/0048540	A1	3/2005	Inami et al.
2005/0058574	A1	3/2005	Bysouth et al.
2005/0084424	A1	4/2005	Ganesan et al.
2005/0106066	A1	5/2005	Saltsman et al.
2005/0121324	A1	6/2005	Park et al.
2005/0133370	A1	6/2005	Park et al.
2005/0135655	A1	6/2005	Kopf-Sill et al.
2005/0152808	A1	7/2005	Ganesan
2005/0170362	A1	8/2005	Wada et al.
2005/0186585	A1	8/2005	Juncosa et al.
2005/0202470	A1	9/2005	Sundberg et al.
2005/0202504	A1	9/2005	Anderson et al.
2005/0208676	A1	9/2005	Kahatt
2005/0220675	A1	10/2005	Reed et al.
2005/0227269	A1	10/2005	Lloyd et al.
2005/0233370	A1	10/2005	Ammann et al.
2005/0238545	A1	10/2005	Parce et al.
2006/0041058	A1	2/2006	Yin et al.
2006/0057039	A1	3/2006	Morse et al.
2006/0057629	A1	3/2006	Kim
2006/0062696	A1	3/2006	Chow et al.
2006/0094108	A1	5/2006	Yoder et al.
2006/0113190	A1	6/2006	Kurnik
2006/0133965	A1	6/2006	Tajima et al.
2006/0134790	A1	6/2006	Tanaka et al.
2006/0148063	A1	7/2006	Fauzzi et al.
2006/0165558	A1	7/2006	Witty et al.
2006/0165559	A1	7/2006	Greenstein et al.
2006/0166233	A1	7/2006	Wu et al.
2006/0177376	A1	8/2006	Tomalia et al.
2006/0177855	A1	8/2006	Utermohlen et al.
2006/0183216	A1	8/2006	Handique et al.
2006/0207944	A1	9/2006	Siddiqi
2006/0246493	A1	11/2006	Jensen et al.
2006/0246533	A1	11/2006	Fathollahi et al.
2007/0004028	A1	1/2007	Lair et al.
2007/0009386	A1	1/2007	Padmanabhan et al.
2007/0020699	A1	1/2007	Carpenter et al.
2007/0026421	A1	2/2007	Sundberg et al.
2007/0042441	A1	2/2007	Masters et al.
2007/0092901	A1	4/2007	Ligler et al.
2007/0098600	A1	5/2007	Kayyem
2007/0104617	A1	5/2007	Coulling et al.
2007/0178607	A1	8/2007	Prober et al.
2007/0292941	A1	12/2007	Handique et al.
2008/0056948	A1	3/2008	Dale et al.
2008/0262213	A1	10/2008	Wu et al.
2009/0131650	A1	5/2009	Brahmasandra et al.
2009/0189089	A1	7/2009	Bedingham et al.
2010/0009351	A1	1/2010	Brahmasandra et al.
2012/0171759	A1	7/2012	Williams et al.
2012/0183454	A1	7/2012	Handique
2012/0258463	A1	10/2012	Duffy et al.

FOREIGN PATENT DOCUMENTS

FR	2795426		12/2000
JP	58212921	A	12/1983
JP	H07-290706		11/1995
JP	2001-509437		7/2001
JP	2001-527220		12/2001
JP	2002-215241		7/2002
JP	2003-500674		1/2003
JP	2005-514718		5/2005
JP	2005-204661		8/2005
JP	2005-291954	A	10/2005
WO	WO 88/06633		9/1988
WO	WO 90/12350		10/1990
WO	WO 92/05443		4/1992
WO	WO 97/05492		2/1997
WO	WO 98/00231		1/1998
WO	WO 98/22625		5/1998
WO	WO 98/53311		11/1998
WO	WO 99/01688		1/1999
WO	WO 99/09042		2/1999
WO	WO 99/12016		3/1999
WO	WO 99/33559		7/1999
WO	WO 01/05510		1/2001
WO	WO 01/14931		3/2001
WO	WO 01/27614		4/2001
WO	WO 01/28684		4/2001
WO	WO 01/41931		6/2001
WO	WO 01/54813		8/2001
WO	WO 01/89681		11/2001
WO	WO 02/072264		9/2002
WO	WO 02/078845		10/2002
WO	WO 03/012325		2/2003
WO	WO 03/012406		2/2003
WO	WO 03/048295		6/2003
WO	WO 03/055605		7/2003
WO	WO 2004/007081		1/2004
WO	WO 2004/074848		9/2004
WO	WO 2005/011867		2/2005
WO	WO 2005/108620		11/2005
WO	WO 2006/079082		7/2006
WO	WO 2007/050327		5/2007
WO	WO 2008/030914		3/2008
WO	WO 2009/012185		1/2009
WO	WO 2010/118541		10/2010

OTHER PUBLICATIONS

International Search Report and Written Opinion, dated Oct. 3, 2008, issued in International Application No. PCT/US2008/69897.

International Search Report, dated Jan. 31, 2006, issued in International Application No. PCT/US2005/15345.

Yoza,et al., "Fully Automated DNA Extraction from Blood Using Magnetic Particles Modified with a Hyperbranched Polyamidomine Dendrimer", Journal of Bioscience and Bioengineering, 95(1):21-26, 2003.

Breadmore, M.C. et al., "Microchip-Based Purification of DNA from Biological Samples", *Anal. Chem.*, vol. 75 (2003), pp. 1880-1886.

Broyles et al., "Sample Filtration, Concentration, and Separation Integrated on Microfluidic Devices", *Analytical Chemistry*, vol. 75, No. 11, pp. 2761-2767 (2003).

Burns et al., "An Integrated Nanoliter DNA Analysis Device", *Science* 282:484-487 (1998).

Chung, Y. et al., "Microfluidic chip for high efficiency DNA extraction", *Miniaturisation for Chemistry, Biology & Bioengineering*, vol. 4, No. 2 (Apr. 2004), pp. 141-147.

Handique et al., "On-Chip Thermopneumatic Pressure for Discrete Drop Pumping", *Anal. Chem*. 73:1831-1838 (2000).

Handique, K. et al., "Microfluidic flow control using selective hydrophobic patterning", *SPIE*, vol. 3224, pp. 185-194 (1997).

Handique, K. et al., "Nanoliter-volume discrete drop injection and pumping in microfabricated chemical analysis systems", Solid-State Sensor and Actuator Workshop (Hilton Head, South Carolina, Jun. 8-11, 1998) pp. 346-349.

Handylab, Inc., Supplementary European Search Report for European Patent Application No. 05745564 dated Jan. 10, 2008, 5 pages.

He, B. et al., "Microfabricated Filters for Microfluidic Analytical Systems", *Analytical Chemistry*, vol. 71, No. 7 (1999), pp. 1464-1468.

Ibrahim, M.S. et al., "Real-Time Microchip PCR for Detecting Single-Base Differences in Viral and Human DNA", *Analytical Chemistry*, vol. 70, No. 9 (1998), pp. 2013-2017.

Kutter, J.P. et al., "Solid Phase Extraction on Microfluidic Devices ", *J. Microcolumn Separations*, vol. 12, No. 2 (2000), pp. 93-97.

Lagally, E.T. et al., "Single-Molecule DNA Amplification and Analysis in an Integrated Microfluidic Device", *Analytical Chemistry*, vol. 73, No. 3 (2001), pp. 565-570.

Livache, T. et al., "Polypyrrole DNA chip on a Silicon Device: Example of Hepatitis C Virus Genotyping", *Analytical Biochemistry*, vol. 255 (1998), pp. 188-194.

Northrup, M.A. et al., "A Miniature Analytical Instrument for Nucleic Acids Based on Micromachined Silicon Reaction Chambers", *Analytical Chemistry*, vol. 70, No. 5 (1998), pp. 918-922.

Oleschuk, R. et al., "Trapping of Bead-Based Reagents within Microfluidic Systems: On-Chip Solid-Phase Extraction and Electrochromatography", *Analytical Chemistry*, vol. 72, No. 3 (2000), pp. 585-590.

Shoffner, M. et al., "Chip PCR. I. Surface passivation of microfabricated silicon-glass chips for PCR", *Nucleic Acids Research*, vol. 24, No. 2 (1996), pp. 375-379.
Smith, K. et al,. "Comparison of Commercial DNA Extraction Kits for Extraction of Bacterial Genomic DNA from Whole-Blood Samples", *Journal of Clinical Microbiology*, vol. 41, No. 6 (Jun. 2003), pp. 2440-2443.
Waters, et al., "Microchip Device for Cell Lysis, Multiplex PCR Amplification, and Electrophoretic Sizing" *Analytical Chemistry*, vol. 70, No. 1, pp. 158-162 (1998).
Weigl, B.H. et al., "Microfluidic Diffusion-Based Separation and Detection", *Science*, vol. 283 (Jan. 15, 1999), pp. 346-347.
File History of the related U.S. Appl. No. 11/281,247, as of Jun. 1, 2010.
File History of related U.S. Appl. No. 11/281,247, for the period of Jun. 2, 2010-Oct. 27, 2010.
Bollet, C. et al., "A simple method for the isolation of chromosomal DNA from Gram positive or acid-fast bacteria", Nucleic Acids Research, vol. 19, No. 8 (1991), p. 1955.
Brahmassandra, et al., On-Chip DNA Detection in Microfabricated Separation Systems, Part of the SPIE Conference on Microfludic Devices and Systems, 1998, Santa Clara, California, vol. 3515, pp. 242-251.
Brody, et al., Diffusion-Based Extraction in a Microfabricated Device, Sensors and Actuators Elsevier, 1997, vol. A58, No. 1, pp. 13-18.
Carlen et al., "Paraffin Actuated Surface Micromachined Valve," in IEEE MEMS 2000 Conference, p. 381-385, Miyazaki, Japan, Jan. 2000.
Handique, K. et al., "Mathematical Modeling of Drop Mixing in a Slit-Type Micochannel", J. Micromech. Microeng., 11:548-554 (2001).
Handique, K. et al., "Nanoliter Liquid Metering in Microchannels Using Hydrophobic Patterns", Anal. Chem., 72:4100-4109 (2000).
Khandurina, et al., Microfabricated Porous Membrane Structure for Sample Concentraction and Electrophoretic Analysis, Analytical Chemistry American Chemical Society, 1999, vol. 71, No. 9, pp. 1815-1819.
Kopp, et al., Chemical Amplification: Continuous-Flow PCR on a Chip, www.sciencemag.org, 1998, vol. 280, pp. 1046-1048.
Orchid BioSciences, Inc., www.orchid.com, Jul. 6, 2001.
Roche, et al. "Ectodermal commitment of insulin-producing cells derived from mouse embryonic stem cells" Faseb J (2005) 19: 1341-1343.
Ross, et al., Analysis of DNA Fragments from Conventional and Microfabricated PCR Devices Using Delayed Extraction MALDI-TOF Mass Spectrometry, Analytical Chemistry, American Chemical Society, 1998, vol. 70, No. 10, pp. 2067-2073.
Yoza, et al., "Fully Automated DNA Extraction fro Blood Using Magnetic Particles Modified with a Hyperbranched Polyamidomine Dendrimer", Journal of Bioscience and Bioengineering, 95(1):21-26, 2003.
Mascini et al., "DNA electrochemical biosensors", Fresenius J. Anal. Chem., 369: 15-22, (2001).
Nakagawa et al., Fabrication of amino silane-coated microchip for DNA extraction from whole blood, J of Biotechnology, Mar. 2, 2005, vol. 116, pp. 105-111.
Plambeck et al., "Electrochemical Studies of Antitumor Antibiotics", J. Electrochem Soc.: Electrochemical Science and Technology (1984), 131(11): 2556-2563.
Wang, "Survey and Summary, from DNA Biosensors to Gene Chips", Nucleic Acids Research, 28(16):3011-3016, (2000).

* cited by examiner

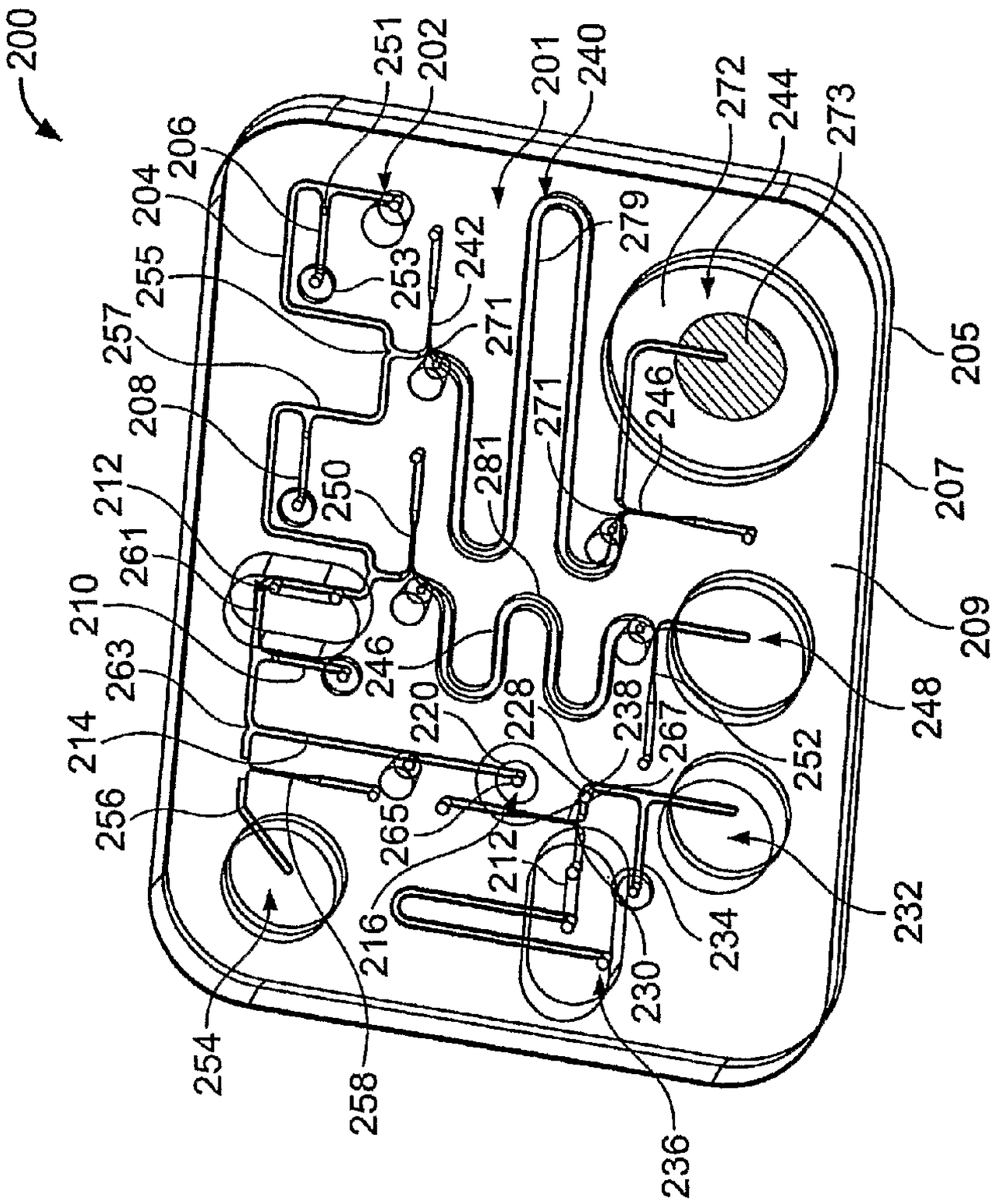

200
251
202
201
240
272
244
273
206
204
255
257
208
212
261
210
263
214
256
254
258
216
265
212
220
228
238
267
252
248
246
250
281
271
279
242
253
271
246
205
207
209
232
234
230
236

FIG. 1

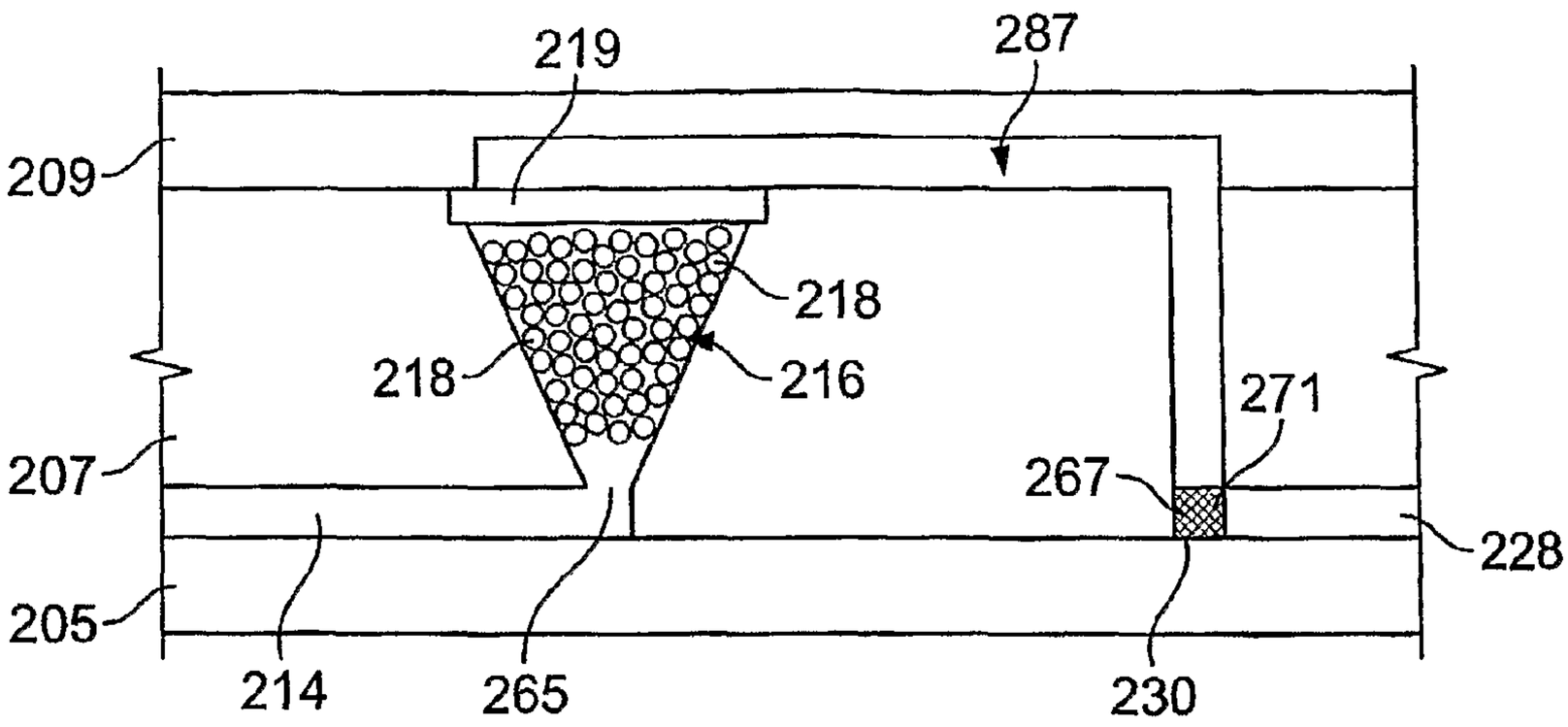

219
287
209
218
218
216
271
207
267
228
205
214
265
230

FIG. 2

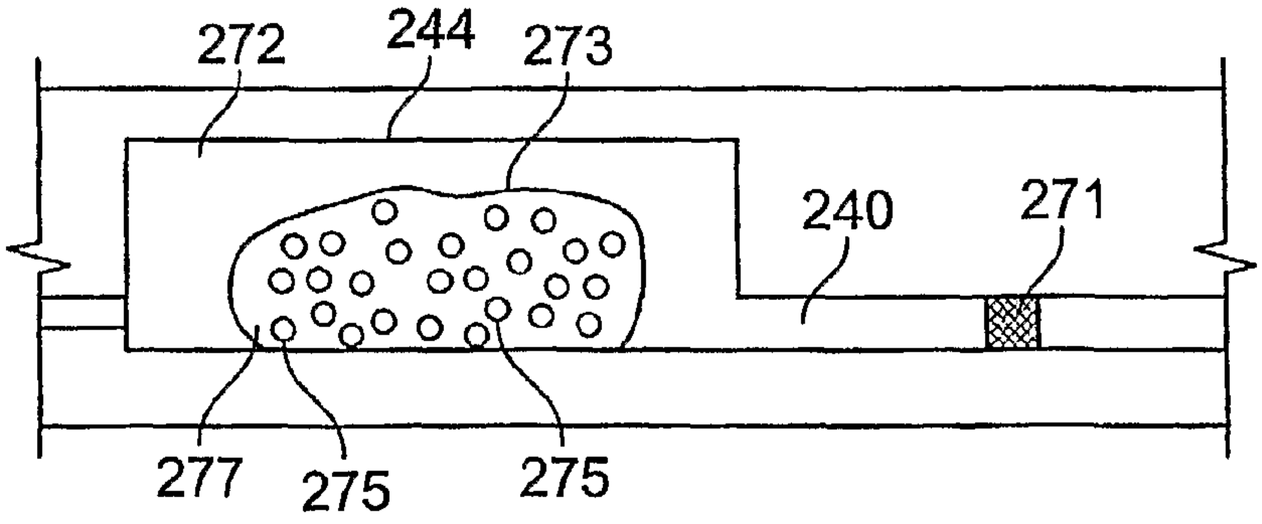

272
244
273
240
271
277
275
275

FIG. 3

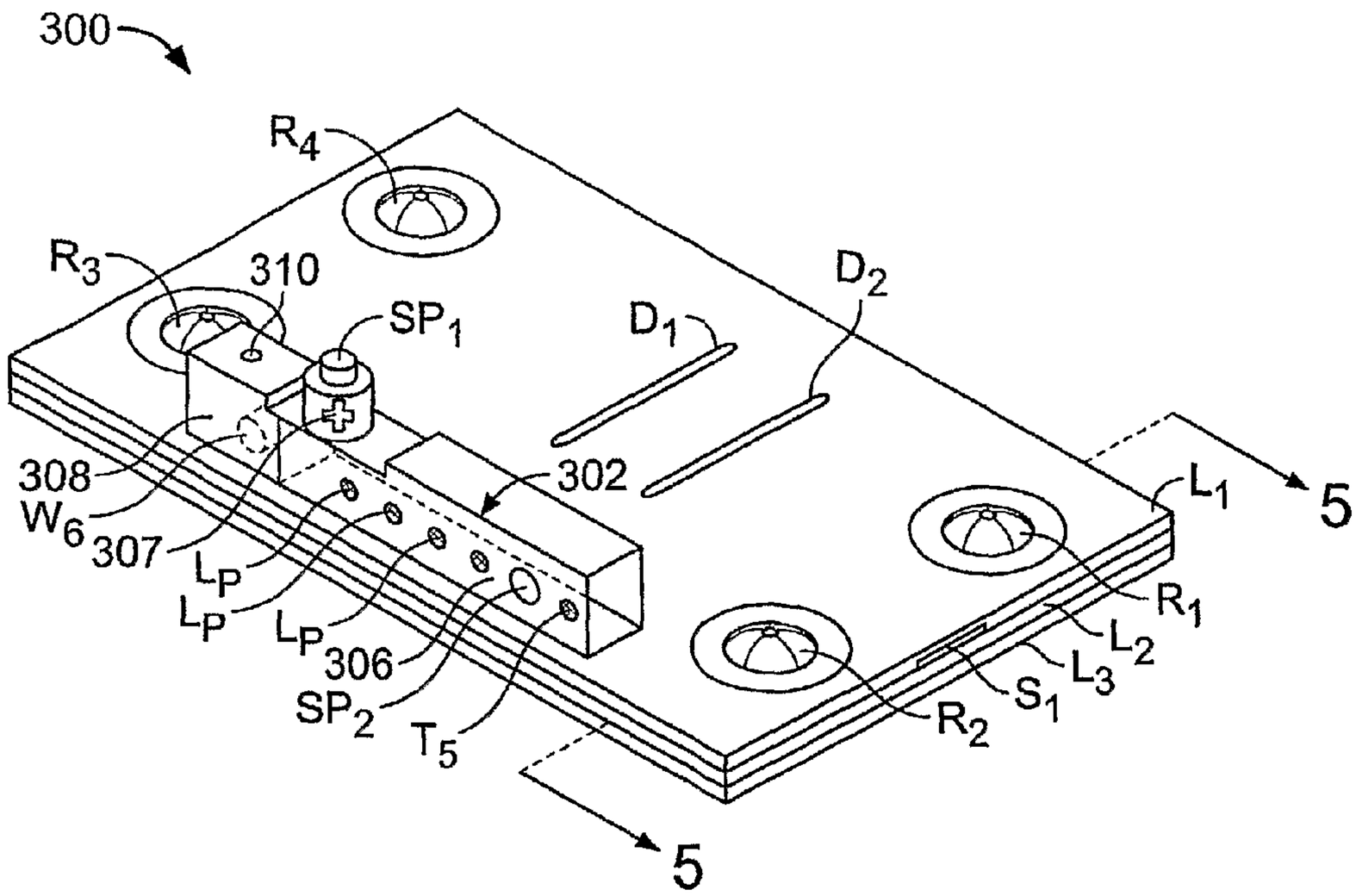

300
R_4
R_3
310
SP_1
D_1
D_2
308
W_6
307
L_P
L_P
L_P
306
SP_2
T_5
302
L_1
5
R_1
L_2
L_3
S_1
R_2
5

FIG. 4

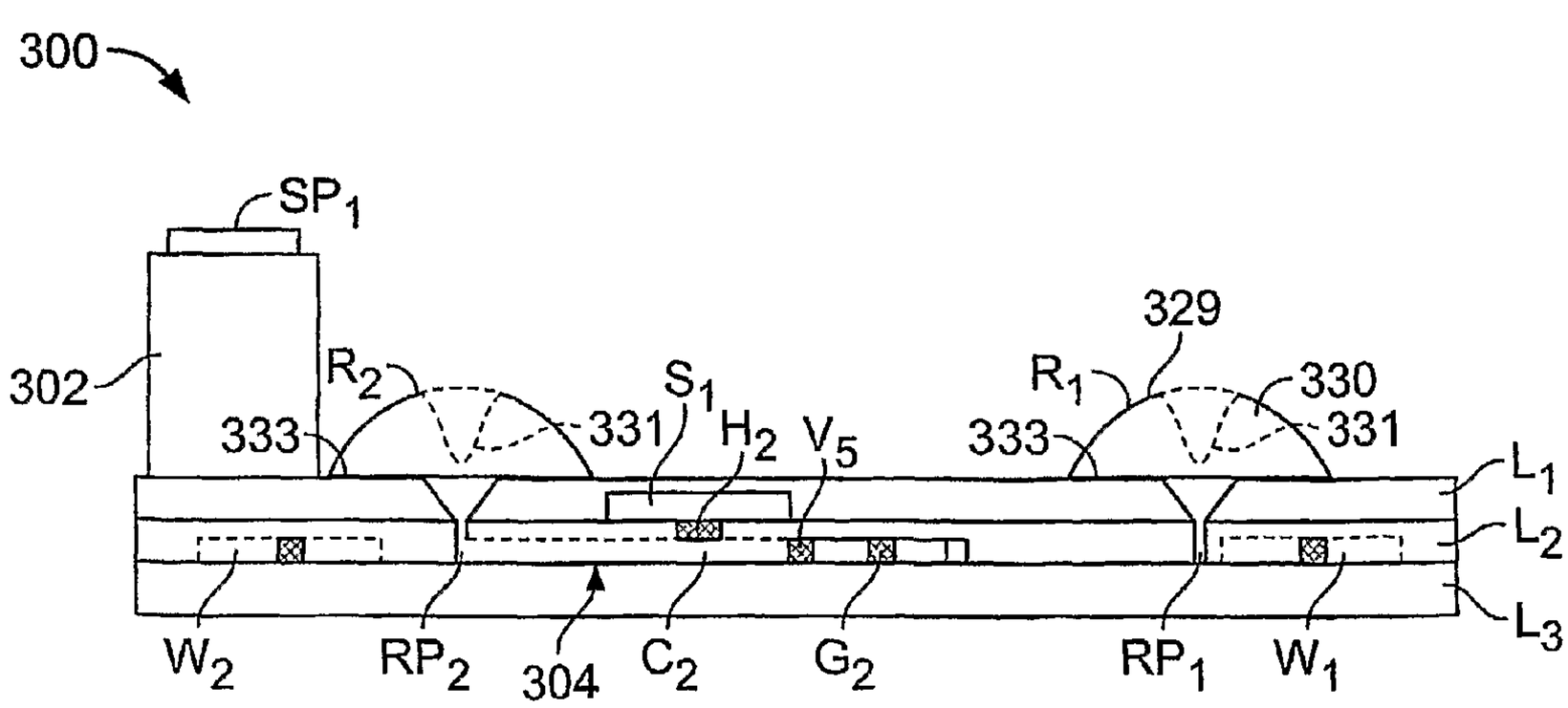

300
SP_1
302
R_2
333
331
S_1
H_2
V_5
329
R_1
330
333
331
L_1
L_2
L_3
W_2
RP_2
304
C_2
G_2
RP_1
W_1

FIG. 5

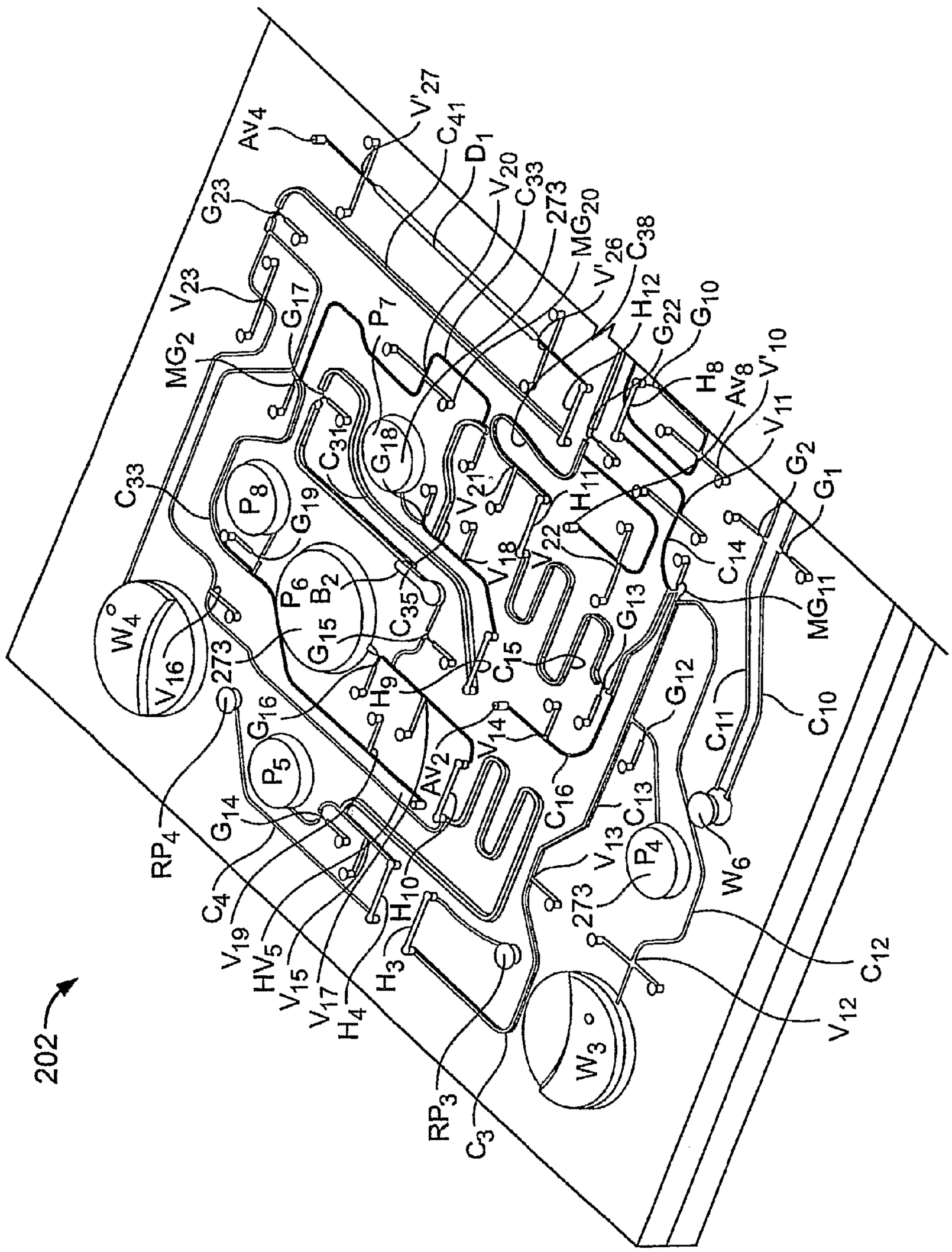

202
W_4
V_{16}
273
RP_4
C_4
G_{14}
P_5
G_{16}
V_{19}
HV_5
V_{15}
V_{17}
H_4
H_{10}
H_3
RP_3
C_3
W_3
V_{12}
C_{12}
W_6
V_{13}
C_{13}
P_4
C_{16}
AV_2
V_{14}
H_9
G_{15}
P_6
B_2
C_{35}
V_{18}
C_{15}
G_{13}
G_{12}
C_{11}
C_{10}
MG_{11}
C_{14}
G_2
G_1
V_{22}
H_{11}
V_{21}
P_8
G_{19}
C_{31}
G_{18}
P_7
MG_2
V_{23}
G_{17}
G_{23}
C_{33}
H_{12}
G_{22}
G_{10}
H_8
AV_8
V'_{10}
V_{11}
V'_{26}
C_{38}
MG_{20}
273
V_{20}
C_{33}
D_1
C_{41}
V'_{27}
AV_4

FIG. 6A

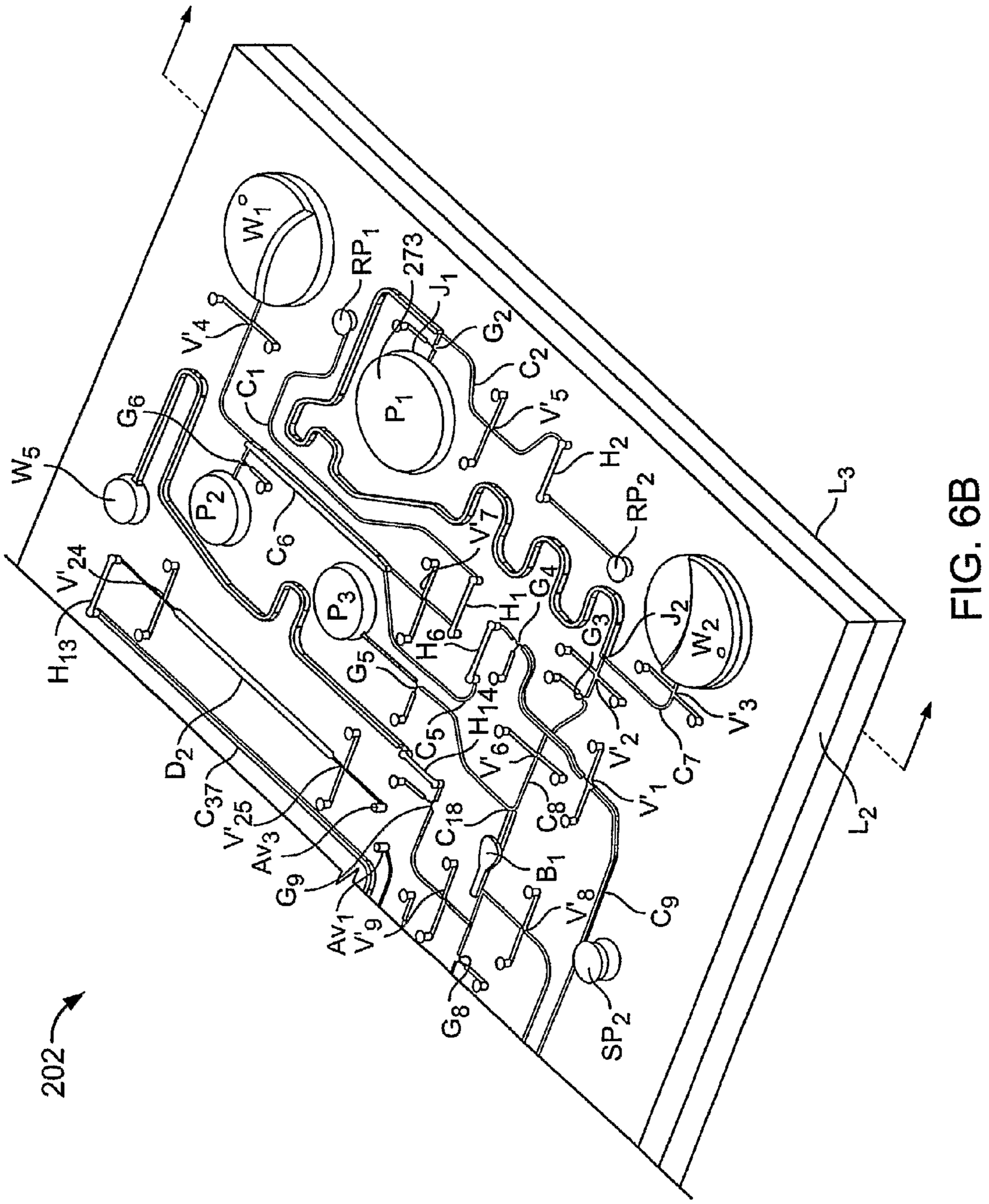

202
W_5
G_6
V'_4
W_1
RP_1
273
J_1
G_2
C_2
V''_5
H_2
RP_2
L_3
L_2
C_1
P_1
P_2
C_6
V''_7
H_1
G_4
J_2
W_2
V'_3
C_7
V'_2
V''_1
C_8
V'_6
H_{14}
C_5
G_5
P_3
H_6
G_3
V'_{24}
H_{13}
D_2
C_{37}
V'_{25}
Av_3
G_9
C_{18}
B_1
V''_8
C_9
Av_1
V'_9
G_8
SP_2

FIG. 6B

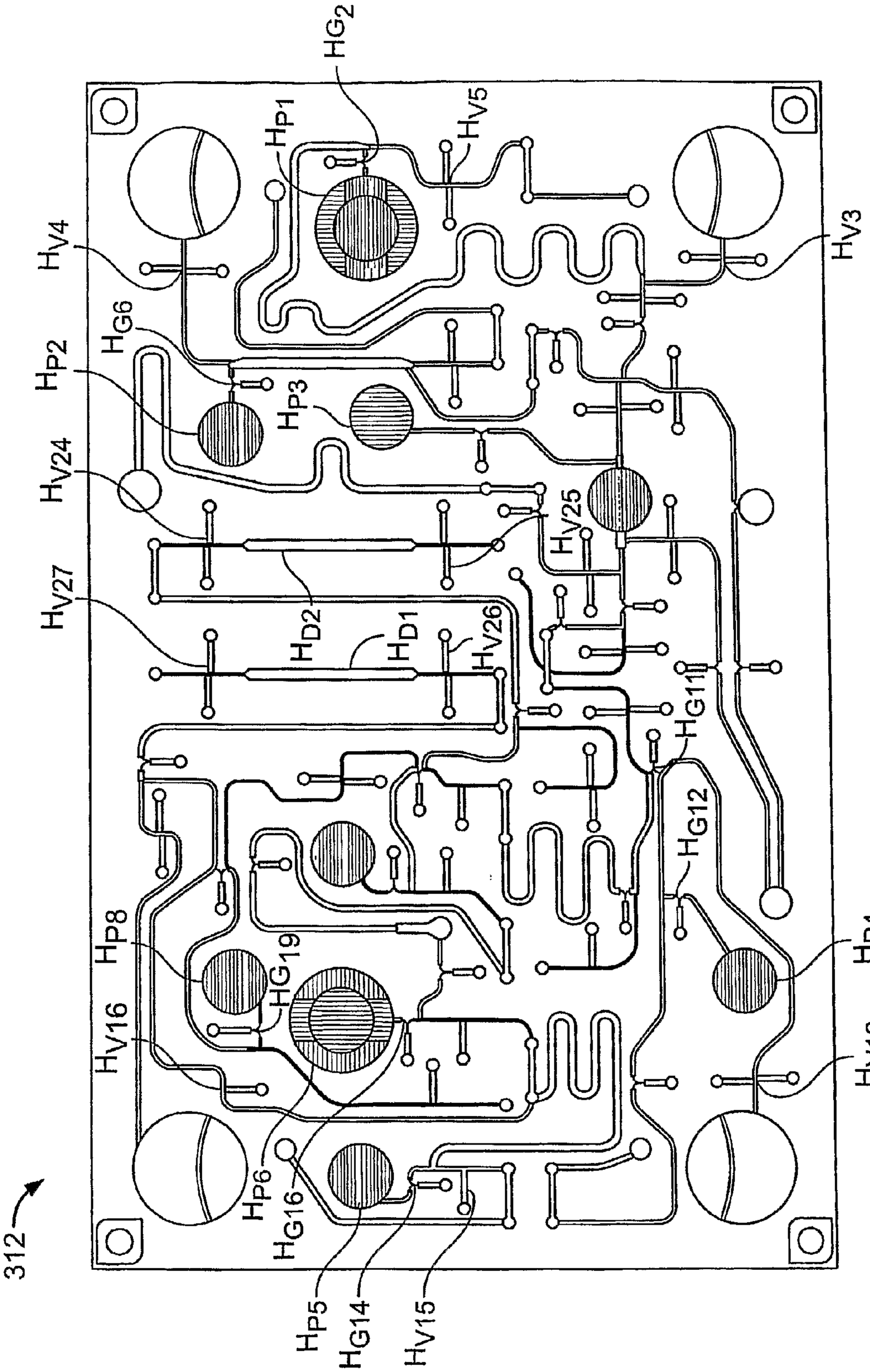

312
HG2
HP1
HV5
HV4
HV3
HG6
HP2
HP3
HV24
HV25
HV27
HD2
HD1
HV26
HG11
HG12
HP8
HG19
HV16
HP4
HV12
HP6
HG16
HP5
HG14
HV15

FIG. 7

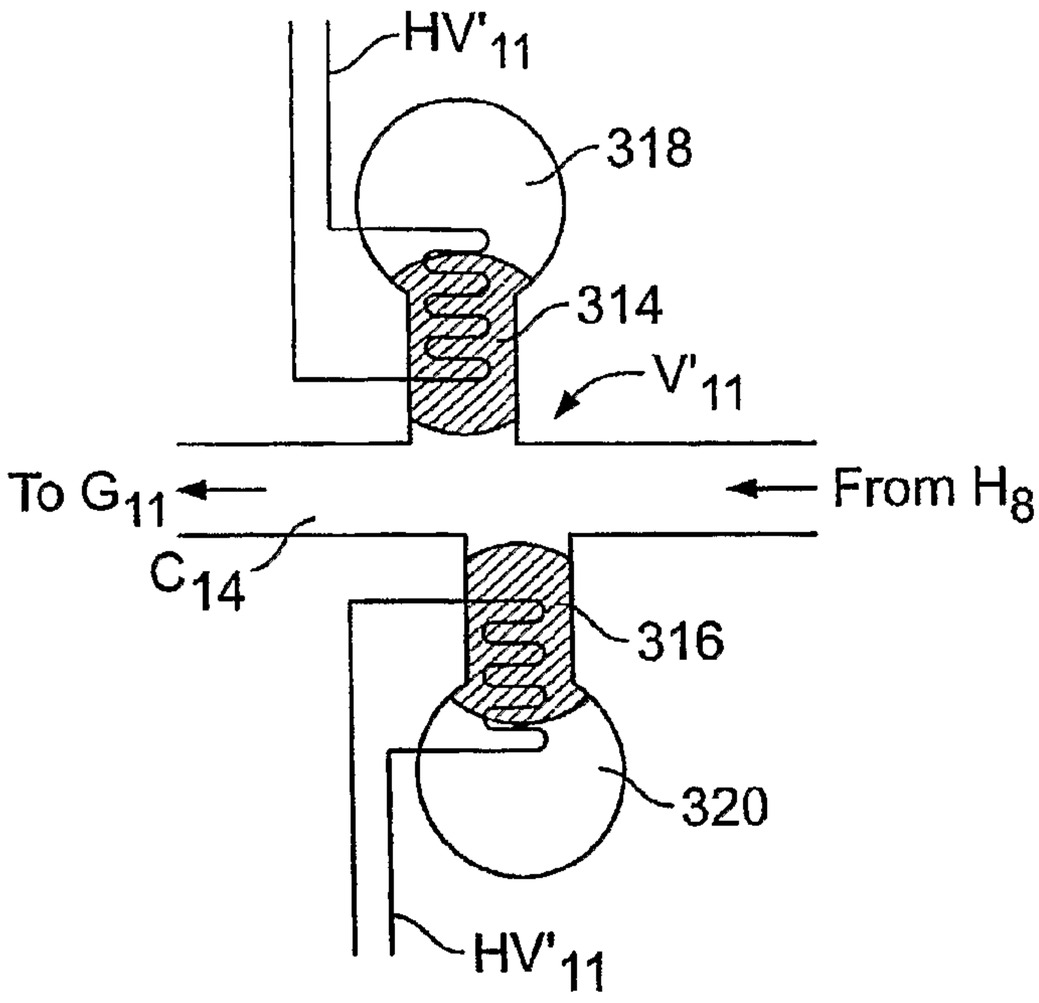

HV'11
318
314
V'11
To G11
From H8
C14
316
320
HV'11

FIG. 8

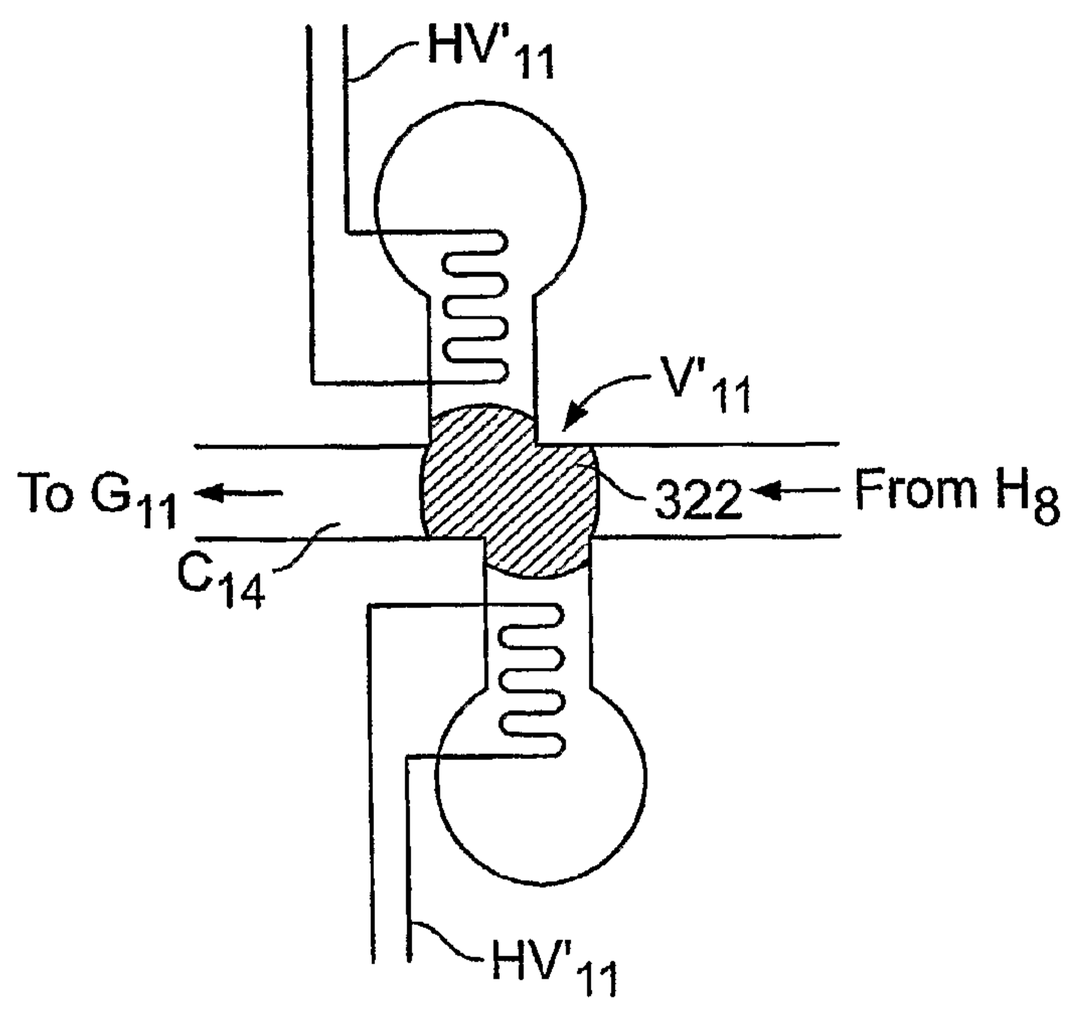

HV'11
V'11
To G11
322
From H8
C14
HV'11

FIG. 9

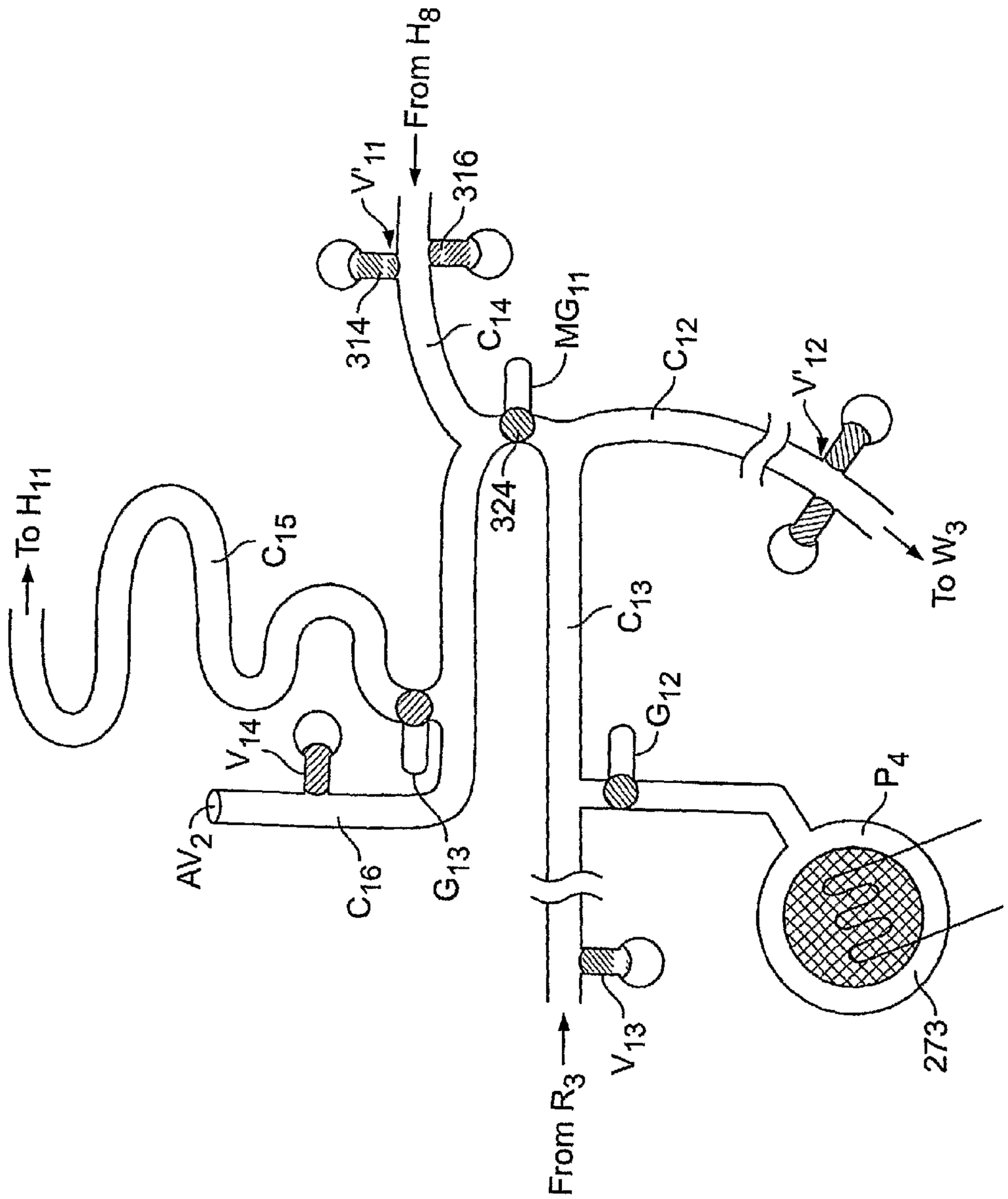

From H_8
V'_{11}
316
314
C_{14}
MG_{11}
C_{12}
V'_{12}
To H_{11}
C_{15}
324
To W_3
C_{13}
V_{14}
G_{12}
P_4
AV_2
C_{16}
G_{13}
From R_3
V_{13}
273

FIG. 10A

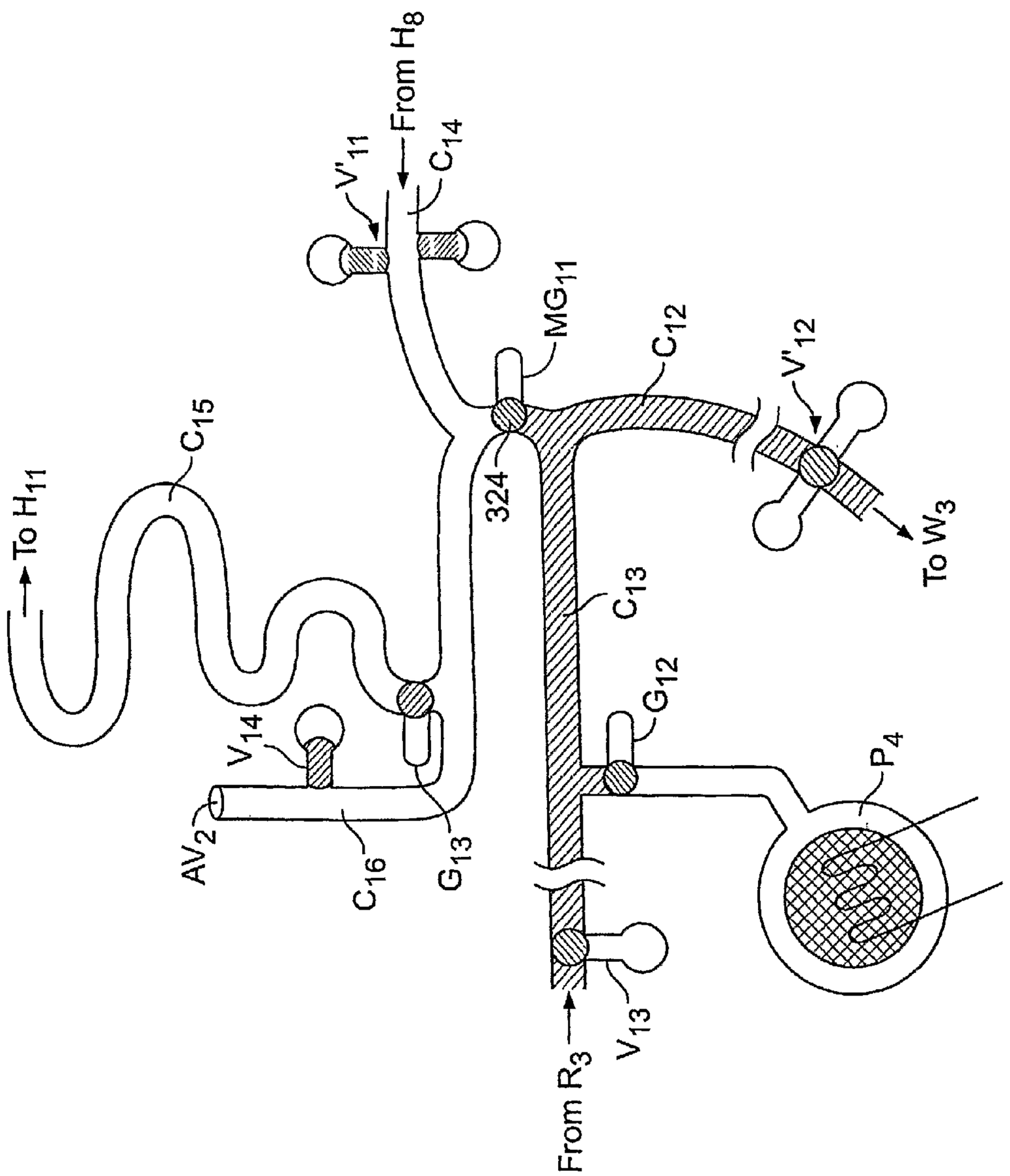

From H_8
V''_{11}
C_{14}
MG_{11}
C_{12}
V''_{12}
To W_3
To H_{11}
C_{15}
324
C_{13}
V_{14}
G_{12}
P_4
AV_2
C_{16}
G_{13}
From R_3
V_{13}

FIG. 10B

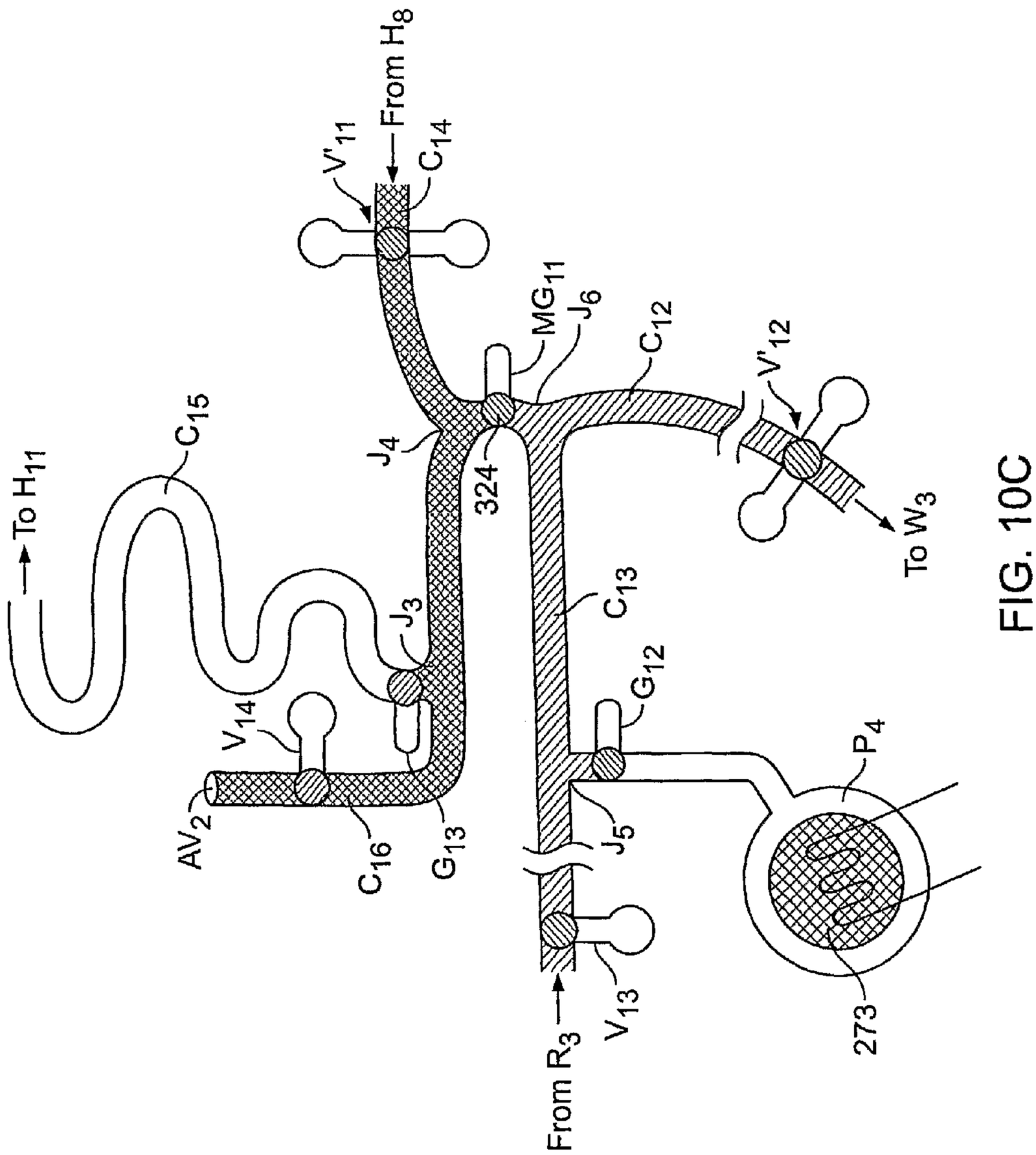

From H_8
V'_{11}
F_{14}
MG_{11}
J_6
C_{12}
V'_{12}
To W_3
To H_{11}
C_{15}
J_4
324
J_3
C_{13}
V_{14}
G_{12}
P_4
AV_2
C_{16}
G_{13}
J_5
From R_3
V_{13}
273

FIG. 10C

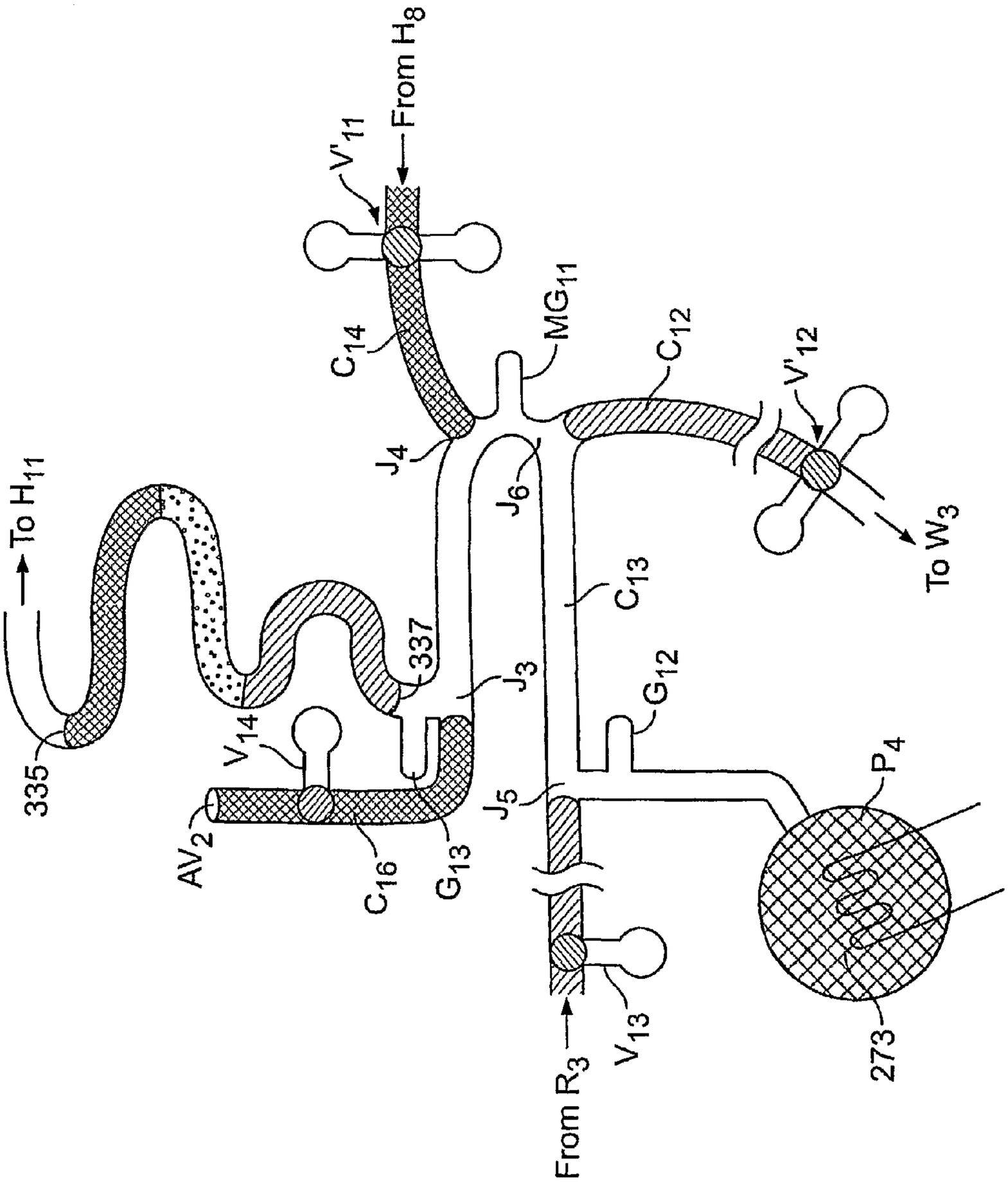

From H_8
V'_{11}
C_{14}
MG_{11}
C_{12}
V'_{12}
To H_{11}
J_4
J_6
To W_3
337
C_{13}
J_3
G_{12}
335
V_{14}
P_4
J_5
AV_2
C_{16}
G_{13}
From R_3
V_{13}
273

FIG. 10D

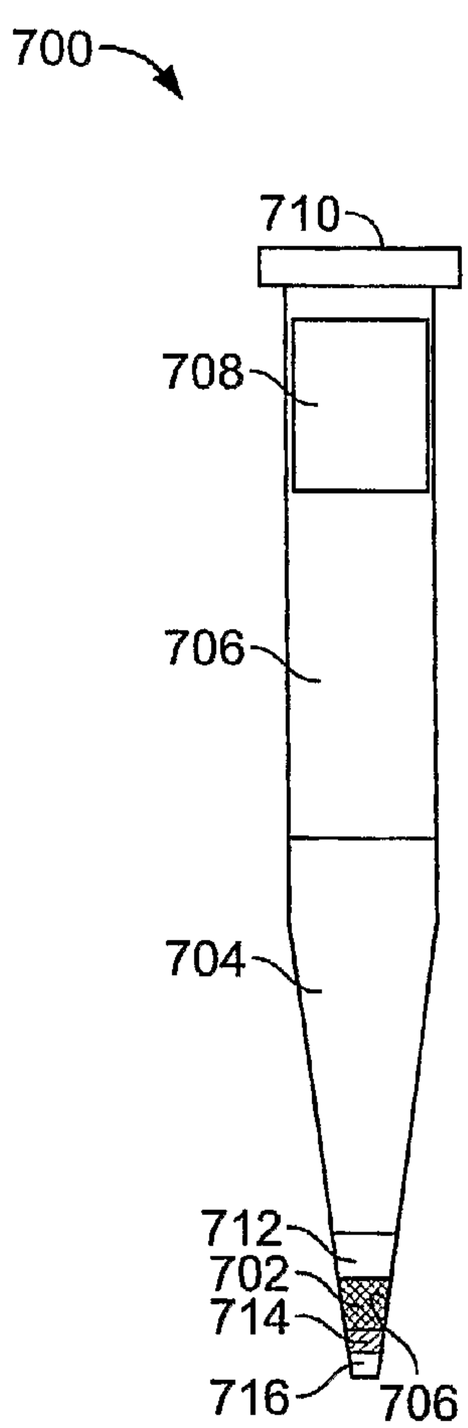


FIG. 11

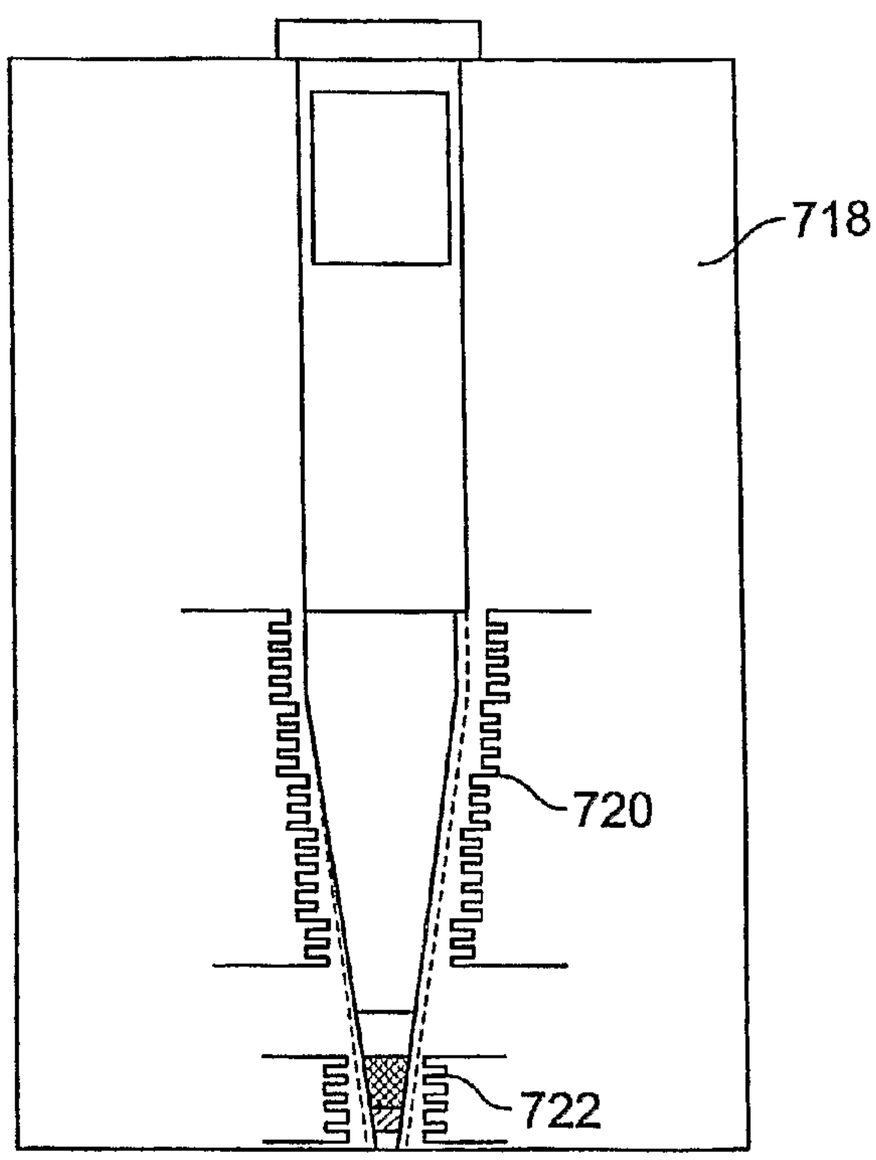


FIG. 12

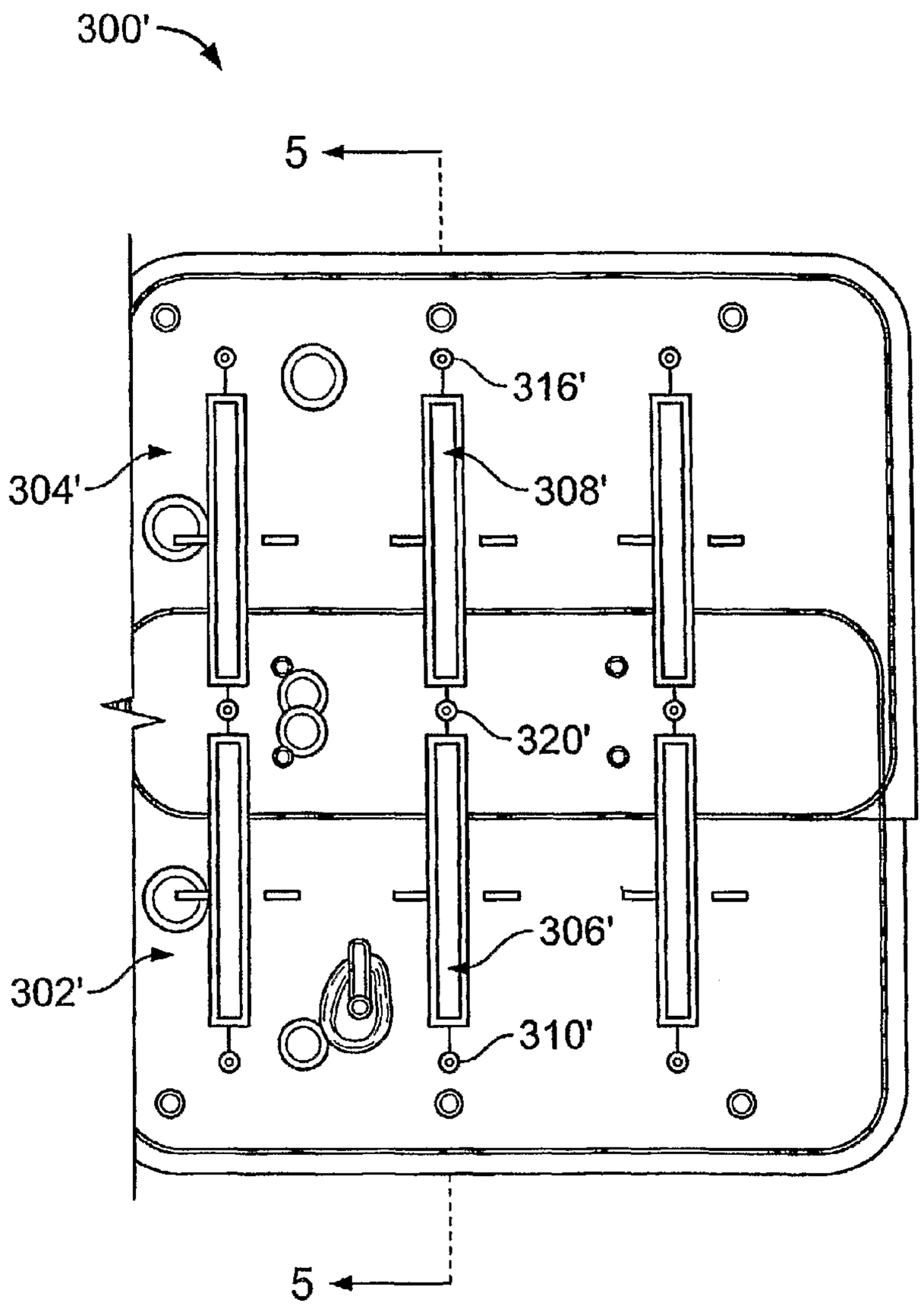


FIG. 13

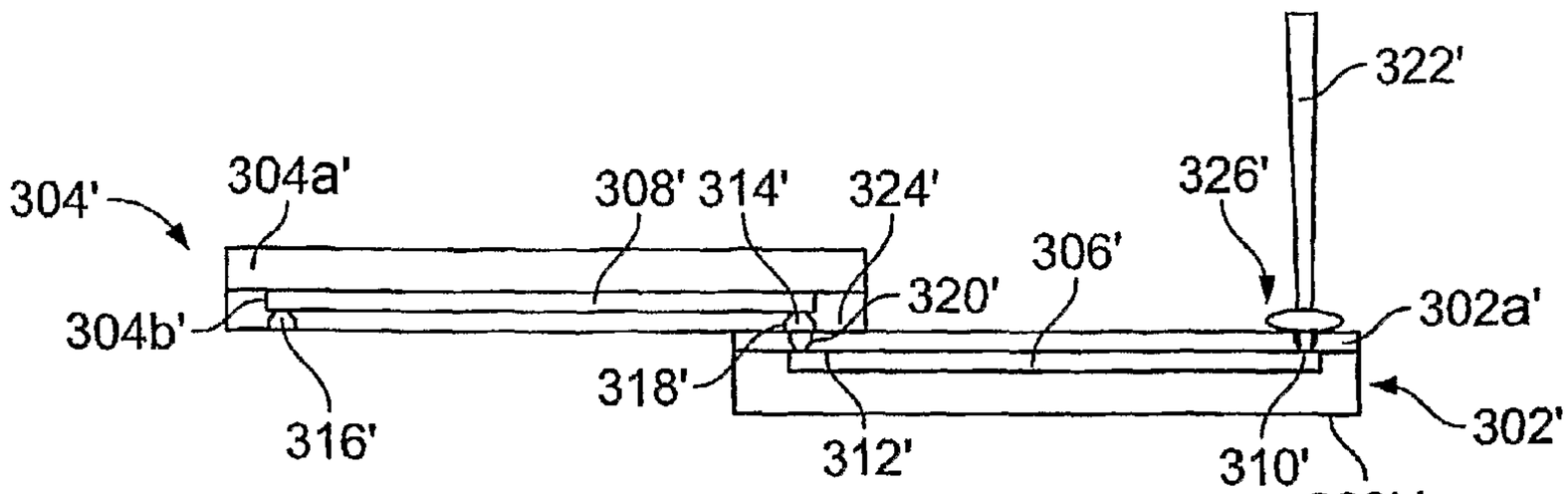

322'
304'
304a'
308' 314'
324'
326'
306'
320'
304b'
302a'
318'
316'
312'
310'
302'
302b'

FIG. 14

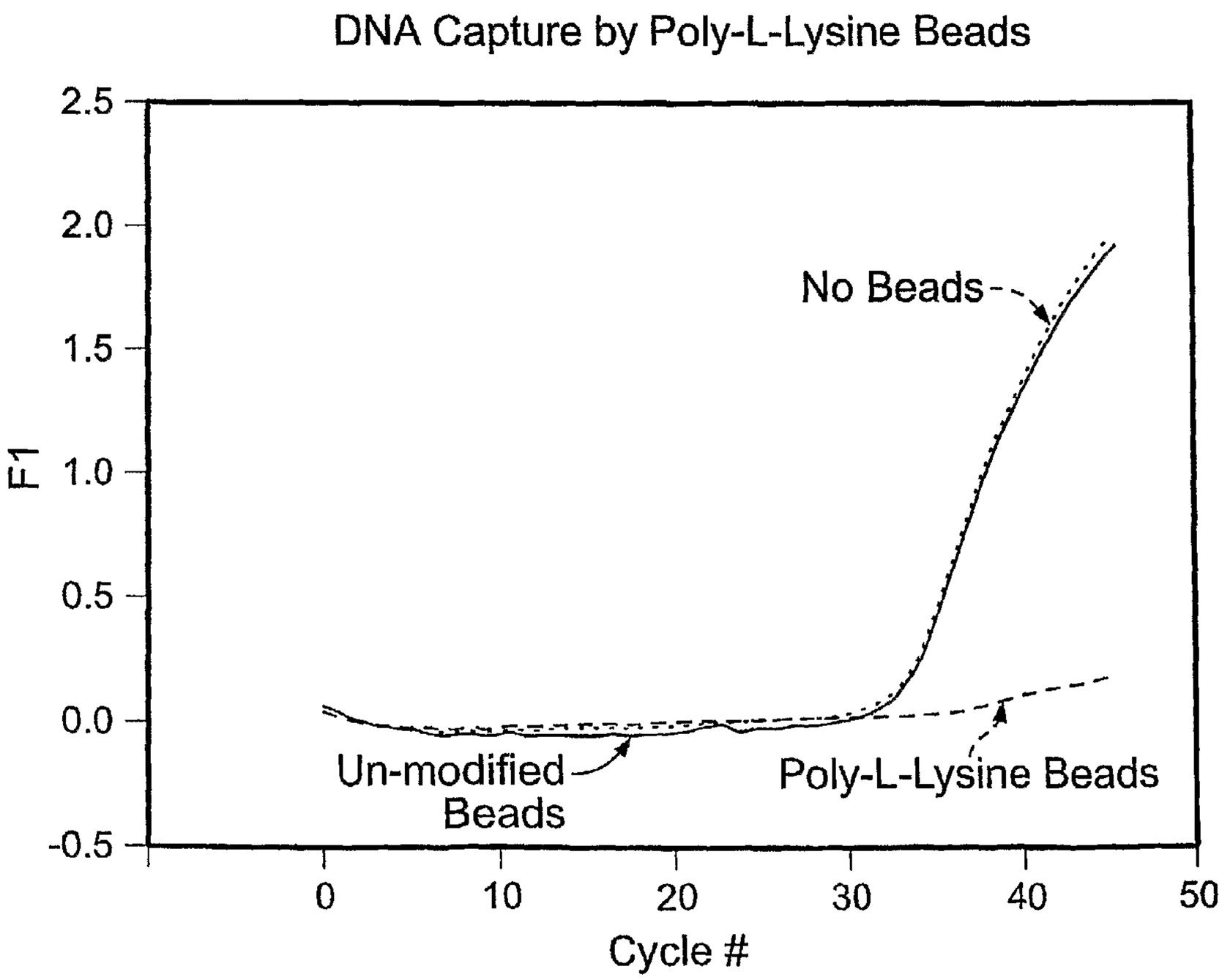

DNA Capture by Poly-L-Lysine Beads
F1
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
0
10
20
30
40
50
Cycle #
No Beads
Un-modified Beads
Poly-L-Lysine Beads

FIG. 15

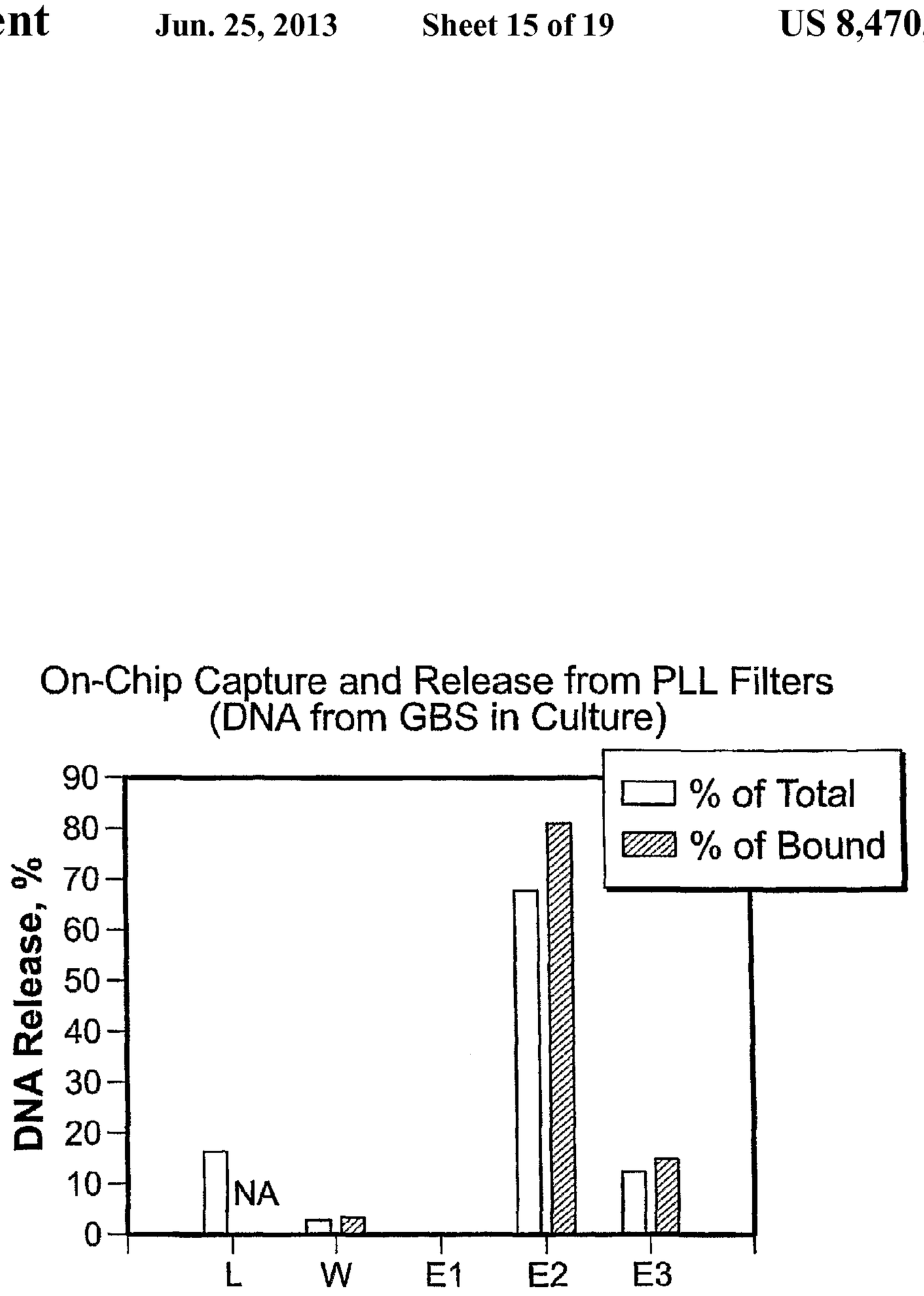

On-Chip Capture and Release from PLL Filters
(DNA from GBS in Culture)
% of Total
% of Bound
DNA Release, %
90
80
70
60
50
40
30
20
10
0
NA
L
W
E1
E2
E3
Fraction

FIG. 16

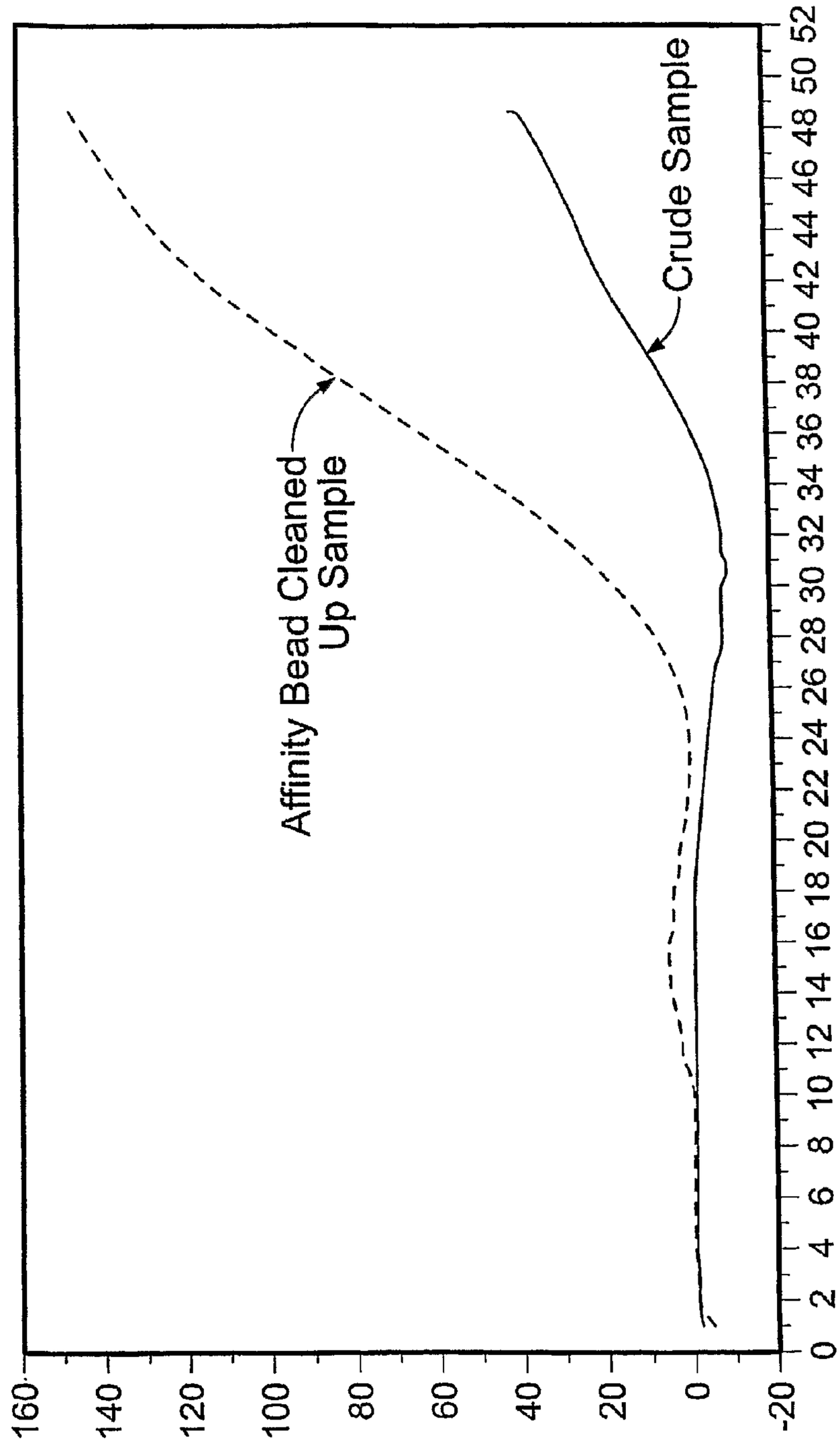

Buccal Sample Wild Type Signal
Affinity Bead Cleaned Up Sample
Crude Sample
PCR Base Line Subtracted CF RFU
Cycle #
160
140
120
100
80
60
40
20
0
-20
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52

FIG. 17

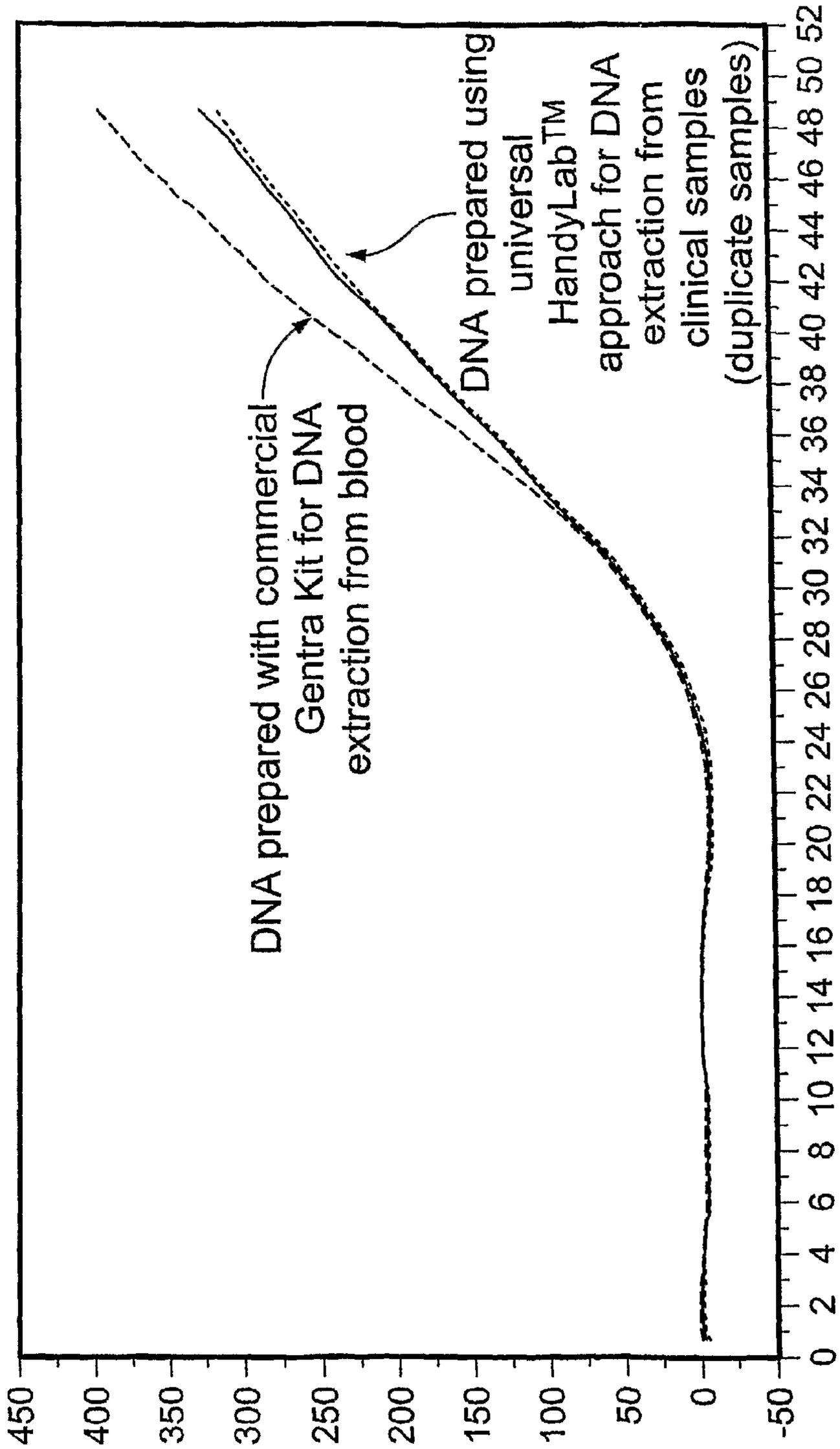

DNA prepared using universal HandyLab™ approach for DNA extraction from clinical samples (duplicate samples)
DNA prepared with commercial-Gentra Kit for DNA extraction from blood
PCR Base Line Subtracted CF RFU
Cycle
450
400
350
300
250
200
150
100
50
0
-50
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52

FIG. 18

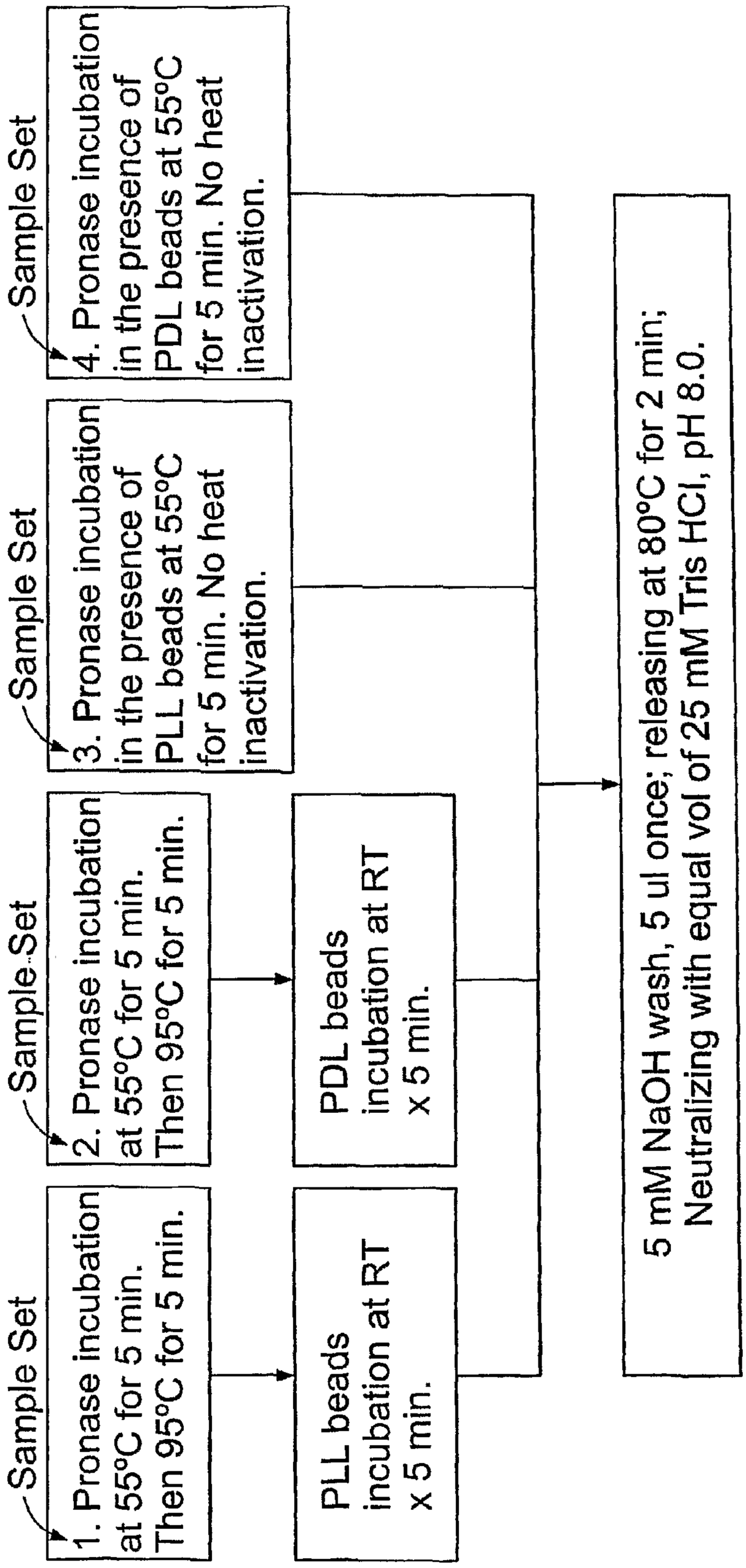

Sample Set
1. Pronase incubation at 55°C for 5 min. Then 95°C for 5 min.
PLL beads incubation at RT x 5 min.
Sample Set
2. Pronase incubation at 55°C for 5 min. Then 95°C for 5 min.
PDL beads incubation at RT x 5 min.
Sample Set
3. Pronase incubation in the presence of PLL beads at 55°C for 5 min. No heat inactivation.
Sample Set
4. Pronase incubation in the presence of PDL beads at 55°C for 5 min. No heat inactivation.
5 mM NaOH wash, 5 ul once; releasing at 80°C for 2 min; Neutralizing with equal vol of 25 mM Tris HCl, pH 8.0.

FIG. 19A

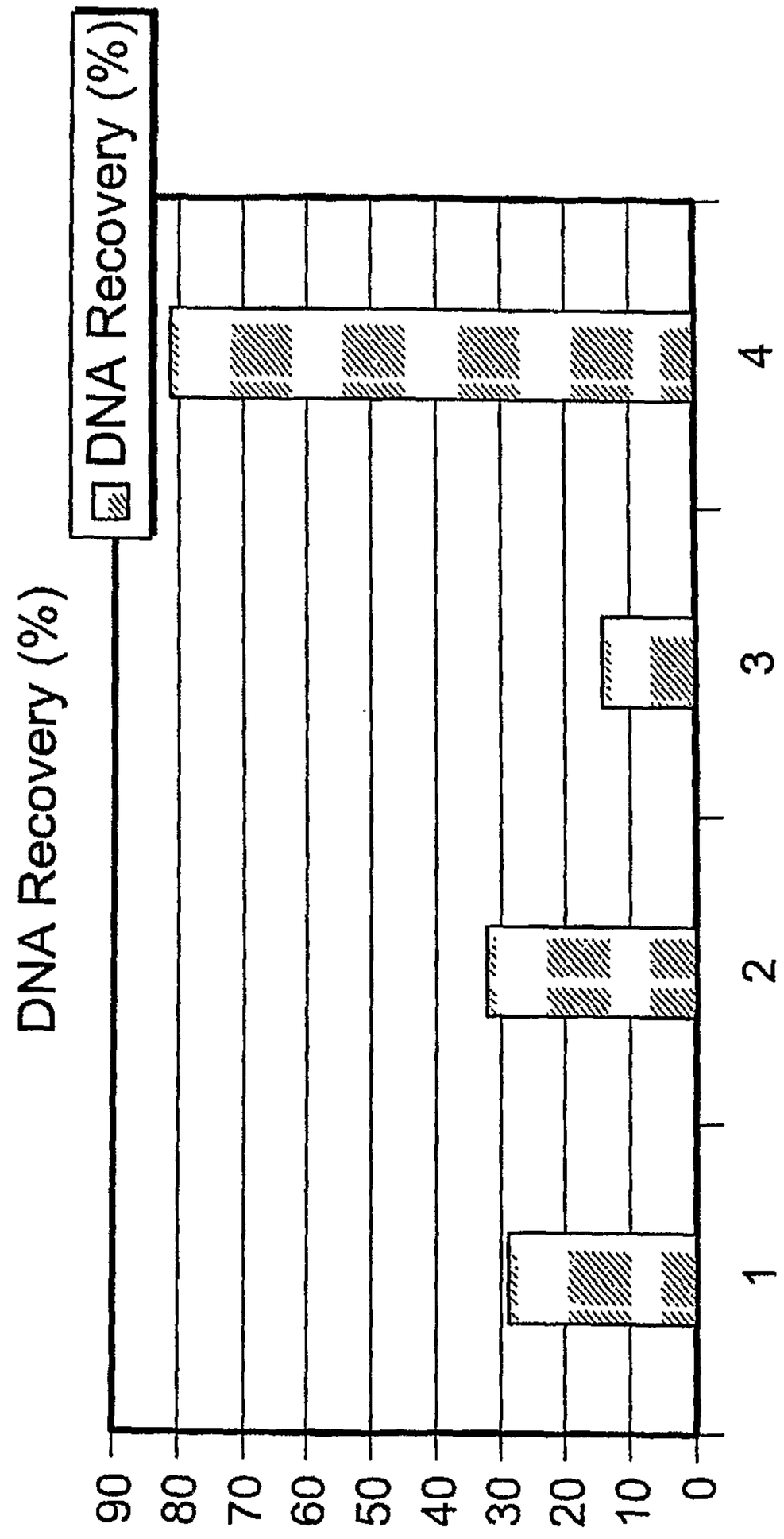


FIG. 19B

PROCESSING POLYNUCLEOTIDE-CONTAINING SAMPLES

RELATED APPLICATIONS

This application claims the benefit of U.S. provisional application No. 60/567,174, filed May 3, 2004 and U.S. provisional application No. 60/645,784, filed Jan. 21, 2005, both of which applications are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

The present invention relates to methods for processing polynucleotide-containing samples as well as to related systems.

BACKGROUND

The analysis of a biological sample often includes detecting one or more polynucleotides present in the sample. One example of detection is qualitative detection, which relates, e.g., to the determination of the presence of the polynucleotide and/or the determination of information related to, e.g., the type, size, presence or absence of mutations, and/or the sequence of the polynucleotide. Another example of detection is quantitative detection, which relates, e.g., to the determination of the amount of polynucleotide present. Detection may include both qualitative and quantitative aspects.

Detecting polynucleotides often involves the use of an enzyme. For example, some detection methods include polynucleotide amplification by polymerase chain reaction (PCR) or a related amplification technique. Other detection methods that do not amplify the polynucleotide to be detected also make use of enzymes. However, the functioning of enzymes used in such techniques may be inhibited by the presence of inhibitors present along with the polynucleotide to be detected. The inhibitors may interfere with, for example, the efficiency and/or specificity of the enzymes.

SUMMARY OF THE INVENTION

One aspect of the present invention relates to a method and related systems for processing one or more polynucleotide(s) (e.g., to concentrate the polynucleotide(s) and/or to separate the polynucleotides from inhibitor compounds (e.g., hemoglobin) that might inhibit detection and/or amplification of the polynucleotides).

In some embodiments, the method includes contacting the polynucleotides and a relatively immobilized compound that preferentially associates with (e.g., retains) the polynucleotides as opposed to inhibitors. An exemplary compound is a poly-cationic polyamide (e.g., poly-L-lysine and/or the poly-D-lysine), which may be bound to a surface (e.g., a surface of one or more particles). The compound retains the polynucleotides so that the polynucleotides and inhibitors may be separated, such as by washing the surface with the compound and associated polynucleotides. Upon separation, the association between the polynucleotide and compound may be disrupted to release (e.g., separate) the polynucleotides from the compound and surface.

In some embodiments, the surface (e.g., a surface of one or more particles) is modified with a poly-cationic polyamide, which may be covalently bound to the surface. The polycationic polyamide may include at least one of poly-L-lysine and poly-D-lysine. In some embodiments, the poly-cationic polyamide (e.g., the at least one of the poly-L-lysine and the poly-D-lysine) have an average molecular weight of at least about 7500 Da. The poly-cationic polyamide (e.g., the at least one of the poly-L-lysine and the poly-D-lysine) may have an average molecular weight of less than about 35,000 Da (e.g., an average molecular weight of less than about 30000 Da (e.g., an average molecular weight of about 25,000 Da)). The poly-cationic polyamide (e.g., the at least one of the poly-L-lysine and the poly-D-lysine) may have a median molecular weight of at least about 15,000 Da. The poly-cationic polyamide (e.g., the at least one of the poly-L-lysine and the poly-D-lysine) may have a median molecular weight of less than about 25,000 Da (e.g., a median molecular weight of less than about 20,000 Da (e.g., a median molecular weight of about 20,000 Da).

Another aspect of the invention relates to a sample preparation device including a surface including a poly-cationic polyamide bound thereto and a sample introduction passage in communication with the surface for contacting the surface with a fluidic sample.

In some embodiments, the device includes a heat source configured to heat an aqueous liquid in contact with the surface to at least about 65° C.

In some embodiments, the device includes a reservoir of liquid having a pH of at least about 10 (e.g., about 10.5 or more). The device is configured to contact the surface with the liquid (e.g., by actuating a pressure source to move the liquid).

In some embodiments, the surface comprises surfaces of a plurality of particles.

In some embodiments, the poly-cationic polyamide includes poly-L-lysine and/or poly-D-lysine.

Another aspect of the invention relates to a method for processing a sample including providing a mixture including a liquid and an amount of polynucleotide, contacting a retention member with the mixture. The retention member may be configured to preferentially retain polynucleotides as compared to polymerase chain reaction inhibitors. Substantially all of the liquid in the mixture is removed from the retention member. The polynucleotides are released from the retention member.

The polynucleotide may have a size of less than about 7.5 Mbp.

The liquid may be a first liquid and removing substantially all of the liquid from the retention member may include contacting the retention member with a second liquid.

Contacting the retention member with a second liquid can include actuating a thermally actuated pressure source to apply a pressure to the second liquid. Contacting the retention member with a second liquid can include opening a thermally actuated valve to place the second liquid in fluid communication with the retention member.

The second liquid may have a volume of less than about 50 microliters.

The retention member may include a surface having a compound configured to bind polynucleotides preferentially to polymerase chain reaction inhibitors (e.g., hemoglobin, peptides, faecal compounds, humic acids, mucousol compounds, DNA binding proteins, or a saccharide).

The surface may include a poly-lysine (e.g., poly-L-lysine and/or poly-D-lysine).

The second liquid may include a detergent (e.g., SDS).

Releasing may include heating the retention member to a temperature of at least about 50° C. (e.g., at about 65° C.). The temperature may be insufficient to boil the liquid in the presence of the retention member during heating. The temperature may be 100° C. or less (e.g., less than 100° C., about 97°

C. or less). The temperature may be maintained for less than about 10 minutes (e.g., for less than about 5 minutes, for less than about 3 minutes).

The releasing may be performed without centrifugation of the retention member.

In certain embodiments, PCR inhibitors are rapidly removed from clinical samples to create a PCR-ready sample. The method may comprise the preparation of a polynucleotide-containing sample that is substantially free of inhibitors. The samples may be prepared from, e.g., crude lysates resulting from thermal, chemical, ultrasonic, mechanical, electrostatic, and other lysing techniques. The samples may be prepared without centrifugation. The samples may be prepared using microfluidic devices or on a larger scale.

Another aspect of the invention relates to a retention member, e.g., a plurality of particles such as beads, comprising bound poly-lysine, e.g., poly-L-lysine, and related methods and systems. The retention member preferentially binds polynucleotides, e.g., DNA, as compared to inhibitors. The retention member may be used to prepare polynucleotides samples for further processing, such as amplification by polymerase chain reaction.

In certain embodiments, more than 90% of a polynucleotide present in a sample may be bound to the retention member, released, and recovered.

In certain embodiments, a polynucleotide may be bound to the retention member, released, and recovered, in less than 10 minutes, less than 7.5 minutes, less than 5 minutes, or less than 3 minutes.

A polynucleotide may be bound to a retention member, released, and recovered without subjecting the polynucleotide, retention member, and/or inhibitors to centrifugation.

Separating the polynucleotides and inhibitors generally excludes subjecting the polynucleotides, inhibitors, processing region, and/or retention member to sedimentation (e.g., centrifugation).

Another aspect of the invention relates to a microfluidic device including a channel, a first mass of a thermally responsive substance (TRS) disposed on a first side of the channel, a second mass of a TRS disposed on a second side of the channel opposite the first side of the channel, a gas pressure source associated with the first mass of the TRS. Actuation of the gas pressure source drives the first mass of the TRS into the second mass of the TRS and obstructs the channel.

The microfluidic device can include a second gas pressure source associated with the second mass of the TRS. Actuation of the second gas pressure source drives the second mass of TRS into the first mass of TRS.

At least one (e.g., both) of the first and second masses of TRS may be a wax.

Another aspect of the invention relates to a method for obstructing a channel of a microfluidic device. A mass of a TRS is heated and driven across the channel (e.g., by gas pressure) into a second mass of TRS. The second mass of TRS may also be driven (e.g., by gas pressure) toward the first mass of TRS.

Another aspect of the invention relates to an actuator for a microfluidic device. The actuator includes a channel, a chamber connected to the channel, at least one reservoir of encapsulated liquid disposed in the chamber, and a gas surrounding the reservoir within the chamber. Heating the chamber expands the reservoir of encapsulated liquid and pressurizes the gas. Typically the liquid has a boiling point of about 90° C. or less. The liquid may be a hydrocarbon having about 10 carbon atoms or fewer.

The liquid may be encapsulated by a polymer.

The actuator may include multiple reservoirs of encapsulated liquid disposed in the chamber.

The multiple reservoirs may be dispersed within a solid (e.g., a wax).

The multiple reservoirs may be disposed within a flexible enclosure (e.g., a flexible sack).

Another aspect of the invention relates to a method including pressurizing a gas within a chamber of a microfluidic to create a gas pressure sufficient to move a liquid within a channel of the microfluidic device. Pressurizing the gas typically expanding at least one reservoir of encapsulated liquid disposed within the chamber.

Expanding the at least one reservoir can include heating the chamber.

Pressurizing the gas can include expanding multiple reservoirs of encapsulated liquid.

Another aspect of the invention relates to a method for combining (e.g., mixing) first and second liquids and related devices. The device includes a mass of a temperature responsive substance (TRS) that separates first and second channels of the device. The device is configured to move a first liquid along the first channel so that a portion (e.g., a medial portion) of the first liquid is adjacent the TRS and to move a second liquid along the second channel so that a portion (e.g., a medial portion) of second liquid is adjacent the TRS. A heat source is actuated to move the TRS (e.g., by melting, dispersing, fragmenting). The medial portions of the first and second liquids typically combine without being separated by a gas interface. Typically, only a subset of the first liquid and a subset of the second liquid are combined. The liquids mix upon being moved along a mixing channel.

Another aspect of the invention relates to a lyophilized reagent particle and a method of making the particle.

In some embodiments, the lyophilized particles include multiple smaller particles each having a plurality of ligands that preferentially associate with polynucleotides as compared to PCR inhibitors. The lyophilized particles can also (or alternatively) include lysing reagents (e.g., enzymes) configured to lyse cells to release polynucleotides. The lyophilized particles can also (or alternatively) include enzymes (e.g., proteases) that degrade proteins.

Cells can be lysed by combining a solution of the cells with the lyophilized particles to reconstitute the particles. The reconstituted lysing reagents lyse the cells. The polynucleotides associate with ligands of the smaller particles. During lysis, the solution may be heated (e.g., radiatively using a lamp (e.g., a heat lamp).

In some embodiments, lyophilized particles include reagents (e.g., primers, control plasmids, polymerase enzymes) for performing a PCR reaction.

A method for making lyophilized particles includes forming a solution of reagents of the particle and a cryoprotectant (e.g., a sugar or poly-alcohol). The solution is deposited dropwise on a chilled hydrophobic surface (e.g., a diamond film or polytetrafluoroethylene surface). The particles freeze and are subjected to reduced pressure (typically while still frozen) for a time sufficient to remove (e.g., sublimate) the solvent. The lyophilized particles may have a diameter of about 5 mm or less (e.g., about 2.5 mm or less, about 1.75 mm or less).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a perspective view of a microfluidic device.

FIG. **2** is a cross-sectional view of a processing region for retaining polynucleotides and/or separating polynucleotides from inhibitors.

FIG. **3**. is a cross-sectional view of an actuator.

FIG. **4** is a perspective view of a microfluidic device.

FIG. **5** is a side cross-sectional view of the microfluidic device of FIG. **4**.

FIG. **6** is a perspective view of a microfluidic network of the microfluidic device of FIG. **4**.

FIG. **7** illustrates an array of heat sources for operating components of the microfluidic device of FIG. **4**.

FIGS. **8** and **9** illustrate a valve in the open and closed states respectively.

FIGS. **10**A-**10**D illustrate a mixing gate of the microfluidic network of FIG. **6** and adjacent regions of the network.

FIG. **11** illustrates a device for separating polynucleotides and inhibitors.

FIG. **12** illustrates the device of FIG. **11** and a device for operation thereof.

FIG. **13** illustrates a microfluidic device.

FIG. **14** is a cross-section of the microfluidic device of FIG. **13** taken along **5**.

FIG. **15** illustrates the retention of herring sperm DNA.

FIG. **16** illustrates the retention and release of DNA from group B streptococci;

FIG. **17** illustrates the PCR response of a sample from which inhibitors had been removed and of a sample from which inhibitors had not been removed.

FIG. **18** illustrates the PCR response of a sample prepared in accord with the invention and a sample prepared using a commercial DNA extraction method.

FIG. **19***a* illustrates a flow chart showing steps performed during a method for separation polynucleotides and inhibitors.

FIG. **19***b* illustrates DNA from samples subjected to the method of FIG. **19***a*.

DETAILED DESCRIPTION OF THE INVENTION

Analysis of biological samples often includes determining whether one or more polynucleotides (e.g., a DNA, RNA, mRNA, or rRNA) is present in the sample. For example, one may analyze a sample to determine whether a polynucleotide indicative of the presence of a particular pathogen is present. Typically, biological samples are complex mixtures. For example, a sample may be provided as a blood sample, a tissue sample (e.g., a swab of, for example, nasal, buccal, anal, or vaginal tissue), a biopsy aspirate, a lysate, as fungi, or as bacteria. Polynucleotides to be determined may be contained within particles (e.g., cells (e.g., white blood cells and/or red blood cells), tissue fragments, bacteria (e.g., gram positive bacteria and/or gram negative bacteria), fungi, spores). One or more liquids (e.g., water, a buffer, blood, blood plasma, saliva, urine, spinal fluid, or organic solvent) is typically part of the sample and/or is added to the sample during a processing step.

Methods for analyzing biological samples include providing a biological sample (e.g., a swab), releasing polynucleotides from particles (e.g., bacteria) of the sample, amplifying one or more of the released polynucleotides (e.g., by polymerase chain reaction (PCR)), and determining the presence (or absence) of the amplified polynucleotide(s) (e.g., by fluorescence detection). Biological samples, however, typically include inhibitors (e.g., mucousal compounds, hemoglobin, faecal compounds, and DNA binding proteins) that can inhibit determining the presence of polynucleotides in the sample. For example, such inhibitors can reduce the amplification efficiency of polynucleotides by PCR and other enzymatic techniques for determining the presence of polynucleotides. If the concentration of inhibitors is not reduced relative to the polynucleotides to be determined, the analysis can produce false negative results.

We describe methods and related systems for processing biological samples (e.g., samples having one or more polynucleotides to be determined). Typically, the methods and systems reduce the concentration of inhibitors relative to the concentration of polynucleotides to be determined.

Referring to FIG. **1**, a microfluidic device **200** includes first, second, and third layers **205**, **207**, and **209** that define a microfluidic network **201** having various components configured to process a sample including one or more polynucleotides to be determined. Device **200** typically processes the sample by increasing the concentration of a polynucleotide to be determined and/or by reducing the concentration of inhibitors relative to the concentration of polynucleotide to be determined.

We now discuss the arrangement of components of network **201**.

Network **201** includes an inlet **202** by which sample material can be introduced to the network and an output **236** by which a processed sample can be removed (e.g., expelled by or extracted from) network **201**. A channel **204** extends between inlet **202** and a junction **255**. A valve **205** is positioned along channel **204**. A reservoir channel **240** extends between junction **255** and an actuator **244**. Gates **242** and **246** are positioned along channel **240**. A channel **257** extends between junction **255** and a junction **257**. A valve **208** is positioned along channel **257**. A reservoir channel **246** extends between junction **259** and an actuator **248**. Gates **250** and **252** are positioned along channel **246**. A channel **261** extends between junction **259** and a junction **263**. A valve **210** and a hydrophobic vent **212** are positioned along channel **261**. A channel **256** extends between junction **263** and an actuator **254**. A gate **258** is positioned along channel **256**.

A channel **214** extends between junction **263** and a processing chamber **220**, which has an inlet **265** and an outlet **267**. A channel **228** extends between processing chamber outlet **267** and a waste reservoir **232**. A valve **234** is positioned along channel **228**. A channel **230** extends between processing chamber outlet **267** and output **236**.

We turn now to particular components of network **201**.

Referring also to FIG. **2**, processing chamber **220** includes a plurality of particles (e.g., beads, microspheres) **218** configured to retain polynucleotides of the sample under a first set of conditions (e.g., a first temperature and/or first pH) and to release the polynucleotides under a second set of conditions (e.g., a second, higher temperature and/or a second, more basic pH). Typically, the polynucleotides are retained preferentially as compared to inhibitors that may be present in the sample. Particles **218** are configured as a retention member **216** (e.g., a column) through which sample material (e.g., polynucleotides) must pass when moving between the inlet **265** and outlet **267** of processing region **220**.

A filter **219** prevents particles **218** from passing downstream of processing region **220**. A channel **287** connects filter **219** with outlet **267**. Filter **219** has a surface area within processing region **220** that is larger than the cross-sectional area of inlet **265**. For example, in some embodiments, the ratio of the surface area of filter **219** within processing region **220** to the cross-sectional area of inlet **265** (which cross sectional area is typically about the same as the cross-sectional area of channel **214**) is at least about 5 (e.g., at least about 10, at least about 20, at least about 20). In some embodiments, the surface area of filter **219** within processing region **220** is at least about 1 mm^2 (e.g., at least about 2 mm^2, at least about 3 mm^2). In some embodiments, the cross-sectional area of inlet **265** and/or channel **214** is about 0.25 mm^2 or less (e.g.,

about 0.2 mm or less, about 0.15 mm^2 or less, about 0.1 mm^2 or less). The larger surface area presented by filter **219** to material flowing through processing region **220** helps prevent clogging of the processing region while avoiding significant increases in the void volume (discussed below) of the processing region.

Particles **218** are modified with at least one ligand that retains polynucleotides (e.g., preferentially as compared to inhibitors). Typically, the ligands retain polynucleotides from liquids having a pH about 9.5 or less (e.g., about 9.0 or less, about 8.75 or less, about 8.5 or less). As a sample solution moves through processing region **220**, polynucleotides are retained while the liquid and other solution components (e.g., inhibitors) are less retained (e.g., not retained) and exit the processing region. In general, the ligands to release polynucleotides when the pH is about 10 or greater (e.g., about 10.5 or greater, about 11.0 or greater). Consequently, polynucleotides can be released from the ligand modified particles into the surrounding liquid.

Exemplary ligands include, for example, polyamides (e.g., poly-cationic polyamides such as poly-L-lysine, poly-D-lysine, poly-DL-ornithine). Other ligands include, for example, intercalators, poly-intercalators, minor groove binders polyamines (e.g., spermidine), homopolymers and copolymers comprising a plurality of amino acids, and combinations thereof. In some embodiments, the ligands have an average molecular weight of at least about 5000 Da (e.g., at least about 7500 Da, of at least about 15000 Da). In some embodiments, the ligands have an average molecular weight of about 50000 Da or less (e.g., about 35000, or less, about 27500 Da or less). In some embodiments, the ligand is a poly-lysine ligand attached to the particle surface by an amide bond.

In certain embodiments, the ligands are resistant to enzymatic degradation, such as degradation by protease enzymes (e.g., mixtures of endo- and exo-proteases such as pronase) that cleave peptide bonds. Exemplary protease resistant ligands include, for example, poly-D-lysine and other ligands that are enantiomers of ligands susceptible to enzymatic attack.

Particles **218** are typically formed of a material to which the ligands can be associated. Exemplary materials from which particles **218** can be formed include polymeric materials that can be modified to attach a ligand. Typical polymeric materials provide or can be modified to provide carboxylic groups and/or amino groups available to attach ligands. Exemplary polymeric materials include, for example, polystyrene, latex polymers (e.g., polycarboxylate coated latex), polyacrylamide, polyethylene oxide, and derivatives thereof. Polymeric materials that can used to form particles **218** are described in U.S. Pat. No. 6,235,313 to Mathiowitz et al., which patent is incorporated herein by reference Other materials include glass, silica, agarose, and amino-propyl-triethoxy-silane (APES) modified materials.

Exemplary particles that can be modified with suitable ligands include carboxylate particles (e.g., carboxylate modified magnetic beads (Sera-Mag Magnetic Carboxylate modified beads, Part #3008050250, Seradyn) and Polybead carboxylate modified microspheres available from Polyscience, catalog no. 09850). In some embodiments, the ligands include poly-D-lysine and the beads comprise a polymer (e.g., polycarboxylate coated latex).

In general, the ratio of mass of particles to the mass of polynucleotides retained by the particles is no more than about 25 or more (e.g., no more than about 20, no more than about 10). For example, in some embodiments, about 1 gram of particles retains about 100 milligrams of polynucleotides.

Typically, the total volume of processing region **220** (including particles **218**) between inlet **265** and filter **219** is about 15 microliters or less (e.g., about 10 microliters or less, about 5 microliters or less, about 2.5 microliters or less, about 2 microliters or less). In an exemplary embodiment, the total volume of processing region **220** is about 2.3 microliters. In some embodiments, particles **218** occupy at least about 10 percent (e.g., at least about 15 percent) of the total volume of processing region **220**. In some embodiments, particles **218** occupy about 75 percent or less (e.g., about 50 percent or less, about 35 percent or less) of the total volume of processing chamber **220**.

In some embodiments, the volume of processing region **220** that is free to be occupied by liquid (e.g., the void volume of processing region **220** including interstices between particles **218**) is about equal to the total volume minus the volume occupied by the particles. Typically, the void volume of processing region **220** is about 10 microliters or less (e.g., about 7.5 microliters or less, about 5 microliters or less, about 2.5 microliters or less, about 2 microliters or less). In some embodiments, the void volume is about 50 nanoliters or more (e.g., about 100 nanoliters or more, about 250 nanoliters or more). In an exemplary embodiment, the total volume of processing region **220** is about 2.3 microliters. For example, in an exemplary embodiment, the total volume of the processing region is about 2.3 microliters, the volume occupied by particles is about 0.3 microliters, and the volume free to be occupied by liquid (void volume) is about 2 microliters.

Particles **218** typically have an average diameter of about 20 microns or less (e.g., about 15 microns or less, about 10 microns or less). In some embodiments, particles **218** have an average diameter of at least about 4 microns (e.g., at least about 6 microns, at least about 8 microns).

In some embodiments, a volume of channel **287** between filter **219** and outlet **267** is substantially smaller than the void volume of processing region **220**. For example, in some embodiments, the volume of channel **287** between filter **219** and outlet **267** is about 35% or less (e.g., about 25% or less, about 20% or less) of the void volume. In an exemplary embodiment, the volume of channel **287** between filter **219** and outlet **267** is about 500 microliters.

The particle density is typically at least about 10^8 particles per milliliter (e.g., about 10^9 particles per milliliter). For example, a processing region with a total volume of about 1 microliter may include about 10^3 beads.

Filter **219** typically has pores with a width smaller than the diameter of particles **218**. In an exemplary embodiment, filter **219** has pores having an average width of about 8 microns and particles **218** have an average diameter of about 10 microns.

In some embodiments, at least some (e.g., all) of the particles are magnetic. In alternative embodiments, few (e.g., none) of the particles are magnetic.

In some embodiments, at least some (e.g., all) the particles are solid. In some embodiments, at least some (e.g., all) the particles are porous (e.g., the particles may have channels extending at least partially within them).

Channels of microfluidic network **201** typically have at least one sub-millimeter cross-sectional dimension. For example, channels of network **201** may have a width and/or a depth of about 1 mm or less (e.g., about 750 microns or less, about 500 microns, or less, about 250 microns or less).

A valve is a component that has a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). Upon actuation, the valve transitions to a closed state that prevents material from passing along the channel from one

side of the valve to the other. For example, valve **205** includes a mass **251** of a thermally responsive substance (TRS) that is relatively immobile at a first temperature and more mobile at a second temperature. A chamber **253** is in gaseous communication with mass **251**. Upon heating gas (e.g., air) in chamber **253** and heating mass **251** of TRS to the second temperature, gas pressure within chamber **253** moves mass **251** into channel **204** obstructing material from passing therealong. Other valves of network **201** have the same structure and operate in the same fashion as valve **205**.

A mass of TRS can be an essentially solid mass or an agglomeration of smaller particles that cooperate to obstruct the passage. Examples of TRS's include a eutectic alloy (e.g., a solder), wax (e.g., an olefin), polymers, plastics, and combinations thereof. The first and second temperatures are insufficiently high to damage materials, such as polymer layers of device **200**. Generally, the second temperature is less than about 90° C. and the first temperature is less than the second temperature (e.g., about 70° C. or less).

A gate is a component that has a normally closed state that does not allow material to pass along a channel from a position on one side of the gate to another side of the gate. Upon actuation, the gate transitions to a closed state in which material is permitted to pass from one side of the gate (e.g., upstream of the gate) to the other side of the gate (e.g., downstream of the gate). For example, gate **242** includes a mass **271** of TRS positioned to obstruct passage of material between junction **255** and channel **240**. Upon heating mass **271** to the second temperature, the mass changes state (e.g., by melting, by dispersing, by fragmenting, and/or dissolving) to permit passage of material between junction **255** and channel **240**.

The portion of channel **240** between gates **242** and **246** forms a fluid reservoir **279** configured to hold a liquid (e.g., water, an organic liquid, or combination thereof). During storage, gates **242** and **246** limit (e.g., prevent) evaporation of liquid within the fluid reservoir. During operation of device **200**, the liquid of reservoir **279** is typically used as a wash liquid to remove inhibitors from processing region **220** while leaving polynucleotides associated with particles **218**. Typically, the wash liquid is a solution having one or more additional components (e.g., a buffer, chelator, surfactant, a detergent, a base, an acid, or a combination thereof). Exemplary solutions include, for example, a solution of 10-50 mM Tris at pH 8.0, 0.5-2 mM EDTA, and 0.5%-2% SDS, a solution of 10-50 mM Tris at pH 8.0, 0.5 to 2 mM EDTA, and 0.5%-2% Triton X-100.

The portion of channel **246** between gates **250** and **252** form a fluid reservoir **281** configured like reservoir **279** to hold a liquid (e.g., a solution) with limited or no evaporation. During operation of device **200**, the liquid of reservoir **281** is typically used as a release liquid into which polynucleotides that had been retained by particles **218** are released. An exemplary release liquid is an hydroxide solution (e.g., a NaOH solution) having a concentration of, for example, between about 2 mM hydroxide (e.g., about 2 mM NaOH) and about 500 mM hydroxide (e.g., about 500 mM NaOH). In some embodiments, liquid in reservoir **281** is an hydroxide solution having a concentration of about 25 mM or less (e.g., an hydroxide concentration of about 15 mM).

Reservoirs **279**, **281** typically hold at least about 0.375 microliters of liquid (e.g., at least about 0.750 microliters, at least about 1.25 microliters, at least about 2.5 microliters). In some embodiments, reservoirs **279**, **281** hold about 7.5 microliters or less of liquid (e.g., about 5 microliters or less, about 4 microliters or less, about 3 microliters or less).

An actuator is a component that provides a gas pressure that can move material (e.g., sample material and/or reagent material) between one location of network **201** and another location. For example, referring to FIG. **3**, actuator **244** includes a chamber **272** having a mass **273** of thermally expansive material (TEM) therein. When heated, the TEM expands decreasing the free volume within chamber **272** and pressurizing the gas (e.g., air) surrounding mass **273** within chamber **272**. Typically, gates **246** and **242** are actuated with actuator **244**. Consequently, the pressurized gas drives liquid in fluid reservoir **279** towards junction **255**. In some embodiments, actuator **244** can generate a pressure differential of more than about 3 psi (e.g., at least about 4 psi, at least about 5 psi) between the actuator and junction **255**.

The TEM includes a plurality of sealed liquid reservoirs (e.g., spheres) **275** dispersed within a carrier **277**. Typically, the liquid is a high vapor pressure liquid (e.g., isobutane and/or isopentane) sealed within a casing (e.g., a polymeric casing formed of monomers such as vinylidene chloride, acrylonitrile and methylmethacrylate). Carrier **277** has properties (e.g., flexibility and/or an ability to soften (e.g., melt) at higher temperatures) that permit expansion of the reservoirs **275** without allowing the reservoirs to pass along channel **240**. In some embodiments, carrier **277** is a wax (e.g., an olefin) or a polymer with a suitable glass transition temperature. Typically, the reservoirs make up at least about 25 weight percent (e.g., at least about 35 weight percent, at least about 50 weight percent) of the TEM. In some embodiments, the reservoirs make up about 75 weight percent or less (e.g., about 65 weight percent or less, about 50 weight percent or less) of the TEM. Suitable sealed liquid reservoirs can be obtained from Expancel (Akzo Nobel).

When the TEM is heated (e.g., to a temperature of at least about 50° C. (e.g., to at least about 75° C., at least about 90° C.)), the liquid vaporizes and increases the volume of each sealed reservoir and of mass **273**. Carrier **277** softens allowing mass **273** to expand. Typically, the TEM is heated to a temperature of less than about 150° C. (e.g., about 125° C. or less, about 110° C. or less, about 100° C. or less) during actuation. In some embodiments, the volume of the TEM expands by at least about 5 times (e.g., at least about 10 times, at least about 20 times, at least about 30 times).

A hydrophobic vent (e.g., vent **212**) is a structure that permits gas to exit a channel while limiting (e.g., preventing) liquid from exiting the channel. Typically, hydrophobic vents include a layer of porous hydrophobic material (e.g., a porous filter such as a porous hydrophobic membrane from Osmonics) that defines a wall of the channel. As discussed below, hydrophobic vents can be used to position a microdroplet of sample at a desired location within network **201**.

Hydrophobic vents typically have a length of at least about 2.5 mm (e.g., at least about 5 mm, at least about 7.5 mm) along a channel. The length of the hydrophobic vent is typically at least about 5 times (e.g., at least about 10 times, at least about 20 times) larger than a depth of the channel within the hydrophobic vent. For example, in some embodiments, the channel depth within the hydrophobic vent is about 300 microns or less (e.g., about 250 microns or less, about 200 microns or less, about 150 microns or less).

The depth of the channel within the hydrophobic vent is typically about 75% or less (e.g., about 65% or less, about 60% or less) of than the depth of the channel upstream and downstream of the hydrophobic vent. For example, in some embodiments the channel depth within the hydrophobic vent is about 150 microns and the channel depth upstream and downstream of the hydrophobic vent is about 250 microns.

A width of the channel within the hydrophobic vent is typically at least about 25% wider (e.g., at least about 50% wider) than a width of the channel upstream from the vent and downstream from the vent. For example, in an exemplary embodiment, the width of the channel within the hydrophobic vent is about 400 microns and the width of the channel upstream and downstream from the vent is about 250 microns.

Microfluidic device **200** can be fabricated as desired. Typically, layers **205**, **207**, and **209** are formed of a polymeric material. Components of network **201** are typically formed by molding (e.g., by injection molding) layers **207**, **209**. Layer **205** is typically a flexible polymeric material (e.g., a laminate) that is secured (e.g., adhesively and/or thermally) to layer **207** to seal components of network **201**. Layers **207** and **209** may be secured to one another using adhesive.

In use, device **200** is typically thermally associated with an array of heat sources configured to operate the components (e.g., valves, gates, actuators, and processing region **220**) of the device. In some embodiments, the heat sources are integral with an operating system, which operates the device during use. The operating system includes a processor (e.g., a computer) configured to actuate the heat sources according to a desired protocol. Processors configured to operate microfluidic devices are described in U.S. application Ser. No. 09/819,105, filed Mar. 28, 2001, which application is incorporated herein by reference. In other embodiments, the heat sources are integral with the device itself.

Device **200** may be operated as follows. Valves of network **201** are configured in the open state. Gates of network **201** are configured in the closed state. A fluidic sample comprising polynucleotides is introduced to network **201** via inlet **202**. For example, sample can be introduced with a syringe having a Luer fitting. The syringe provides pressure to initially move the sample within network **201**. Sample passes along channels **204**, **257**, **261**, and **214** to inlet **265** of processing region **220**. The sample passes through processing region **220**, exits via outlet **267**, and passes along channel **228** to waste chamber **232**. When the trailing edge (e.g., the upstream liquid-gas interface) of the sample reaches hydrophobic vent **212**, pressure provided by the introduction device (e.g., the syringe) is released from network **201** stopping further motion of the sample.

Typically, the amount of sample introduced is about 500 microliters or less (e.g., about 250 microliters or less, about 100 microliters or less, about 50 microliters or less, about 25 microliters or less, about 10 microliters or less). In some embodiments, the amount of sample is about 2 microliters or less (e.g., of about 0.5 microliters or less).

Polynucleotides entering processing region **220** pass through interstices between the particles **218**. Polynucleotides of the sample contact retention member **216** and are preferentially retained as compared to liquid of the sample and certain other sample components (e.g., inhibitors). Typically, retention member **220** retains at least about 50% of polynucleotides (at least about 75%, at least about 85%, at least about 90%) of the polynucleotides present in the sample that entered processing region **220**. Liquid of the sample and inhibitors present in the sample exit the processing region **220** via outlet **267** and enter waste chamber **232**. Processing region is typically at a temperature of about 50° C. or less (e.g., 30° C. or less) during introduction of the sample.

Processing continues by washing retention member **216** with liquid of reservoir **279** to separate remaining inhibitors from polynucleotides retained by retention member **216**. To wash retention member **216**, valve **206** is closed and gates **242**, **246** of first reservoir **240** are opened. Actuator **244** is actuated and moves wash liquid within reservoir **279** along channels **257**, **261**, and **214**, through processing region **220**, and into waste reservoir **232**. The wash liquid moves sample that may have remained within channels **204**, **257**, **261**, and **214** through the processing region and into waste chamber **232**. Once the trailing edge of the wash liquid reaches vent **212**, the gas pressure generated by actuator **244** is vented and further motion of the liquid is stopped.

The volume of wash liquid moved by actuator **244** through processing region **220** is typically at least about 2 times the void volume of processing region **220** (e.g., at least about 3 times the void volume) and can be about 10 times the void volume or less (e.g., about 5 times the void volume or less). Processing region is typically at a temperature of about 50° C. or less (e.g., 30° C. or less) during washing. Exemplary wash fluids include liquids discussed with respect to reservoirs **279** and **281**.

Processing continues by releasing polynucleotides from retention member **216**. Typically, wash liquid from reservoir **279** is replaced with release liquid (e.g., an hydroxide solution) from reservoir **281** before releasing the polynucleotides. Valve **208** is closed and gates **250**, **252** are opened. Actuator **248** is actuated thereby moving release liquid within reservoir **281** along channels **261**, **214** and into processing region **220** and in contact with retention member **216**. When the trailing edge of release liquid from reservoir **281** reaches hydrophobic vent **212**, pressure generated by actuator **248** is vented stopping the further motion of the liquid. The volume of liquid moved by actuator **248** through processing region **220** is typically at least about equal to the void volume of the processing region **220** (e.g., at least about 2 times the void volume) and can be about 10 times the void volume or less (e.g., about 5 times the void volume or less).

Once retention member **216** with retained polynucleotides has been contacted with liquid from reservoir **281**, a releasing step is typically performed. Typically, the releasing step includes heating release liquid present within processing region **216**. Generally, the liquid is heated to a temperature insufficient to boil liquid in the presence of the retention member. In some embodiments, the temperature is 100° C. or less (e.g., less than 100° C., about 97° C. or less). In some embodiments, the temperature is about 65° C. or more (e.g., about 75° C. or more, about 80° C. or more, about 90° C. or more). In some embodiments, the temperature maintained for about 1 minute or more (e.g., about 2 minutes or more, about 5 minutes or more, about 10 minutes or more). In some embodiments, the temperature is maintained for about 30 minutes (e.g., about 15 minutes or less, about 10 minutes or less, about 5 minutes or less). In an exemplary embodiment, processing region **220** is heated to between about 65 and 90° C. (e.g., to about 70° C.) for between about 1 and 7 minutes (e.g., for about 2 minutes).

The polynucleotides are released into the liquid present in the processing region **220** (e.g., the polynucleotides are typically released into an amount of release liquid having a volume about the same as the void volume of the processing region **220**). Typically, the polynucleotides are released into about 10 microliters or less (e.g., about 5 microliters or less, about 2.5 microliters or less) of liquid.

In certain embodiments, the ratio of the volume of original sample moved through the processing region **220** to the volume of liquid into which the polynucleotides are released is at least about 10 (e.g., at least about 50, at least about 100, at least about 250, at least about 500, at least about 1000). In some embodiments, polynucleotides from a sample having a volume of about 2 ml can be retained within the processing

region, and released into about 4 microliters or less (e.g., about 3 microliters or less, about 2 microliters or less, about 1 microliter or less) of liquid.

The liquid into which the polynucleotides are released typically includes at least about 50% (e.g., at least about 75%, at least about 85%, at least about 90%) of the polynucleotides present in the sample that entered processing region **220**. The concentration of polynucleotides present in the release liquid may be higher than in the original sample because the volume of release liquid is typically less than the volume of the original liquid sample moved through the processing region. For example the concentration of polynucleotides in the release liquid may be at least about 10 times greater (e.g., at least about 25 times greater, at least about 100 times greater) than the concentration of polynucleotides in the sample introduced to device **200**. The concentration of inhibitors present in the liquid into which the polynucleotides are released is generally less than concentration of inhibitors in the original fluidic sample by an amount sufficient to increase the amplification efficiency for the polynucleotides.

The time interval between introducing the polynucleotide containing sample to processing region **220** and releasing the polynucleotides into the release liquid is typically about 15 minutes or less (e.g., about 10 minutes or less, about 5 minutes or less).

Liquid including the released polynucleotides may be removed from the processing region **220** as follows. Valves **210** and **234** are closed. Gates **238** and **258** are opened. Actuator **254** is actuated to generate pressure that moves liquid and polynucleotides from processing region **220**, into channel **230**, and toward outlet **236**. The liquid with polynucleotides can be removed using, for example, a syringe or automated sampling device. Depending upon the liquid in contact with retention member **216** during polynucleotide release, the solution with released polynucleotide may be neutralized with an amount of buffer (e.g., an equal volume of 25-50 mM Tris-HCl buffer pH 8.0).

While releasing the polynucleotides has been described as including a heating step, the polynucleotides may be released without heating. For example, in some embodiments, the liquid of reservoir **281** has an ionic strength, pH, surfactant concentration, composition, or combination thereof that releases the polynucleotides from the retention member.

While the polynucleotides have been described as being released into a single volume of liquid present within processing region **220**, other configurations can be used. For example, polynucleotides may be released with the concomitant (stepwise or continuous) introduction of fluid into and/or through processing region **220**. In such embodiments, the polynucleotides may be released into liquid having a volume of about 10 times or less (e.g., about 7.5 times or less, about 5 times or less, about 2.5 times or less, about 2 times or less) than the void volume of the processing region **220**.

While reservoirs **279**, **281** have been described as holding liquids between first and second gates, other configurations can be used. For example, liquid for each reservoir may be held within a pouch (e.g., a blister pack) isolated from network **201** by an generally impermeable membrane. The pouch is configured so that a user can rupture the membrane driving liquid into reservoirs **279**, **281** where actuators **244**, **248** can move the liquid during use.

While processing regions have been described as having microliter scale dimensions, other dimensions can be used. For example, processing regions with surfaces (e.g., particles) configured to preferentially retain polynucleotides as opposed to inhibitors may have large volumes (e.g., many tens of microliters or more, at least about 1 milliliter or more). In some embodiments, the processing region has a bench-top scale.

While processing region **220** has been described as having a retention member formed of multiple surface-modified particles, other configurations can be used. For example, in some embodiments, processing region **220** includes a retention member configured as a porous member (e.g., a filter, a porous membrane, or a gel matrix) having multiple openings (e.g., pores and/or channels) through which polynucleotides pass. Surfaces of the porous member are modified to preferentially retain polynucleotides. Filter membranes available from, for example, Osmonics, are formed of polymers that may be surface-modified and used to retain polynucleotides within processing region **220**. In some embodiments, processing region **220** includes a retention member configured as a plurality of surfaces (e.g., walls or baffles) through which a sample passes. The walls or baffles are modified to preferentially retain polynucleotides.

While processing region **220** has been described as a component of a microfluidic network, other configurations can be used. For example, in some embodiments, the retention member can be removed from a processing region for processing elsewhere. For example, the retention member may be contacted with a mixture comprising polynucleotides and inhibitors in one location and then moved to another location at which the polynucleotides are removed from the retention member.

While reservoirs **275** have been shown as dispersed within a carrier, other configurations may be used. For example, reservoirs **275** can be encased within a flexible enclosure formed by a, for example, (e.g., a membrane, for example, an enclosure such as a sack). In some embodiments, reservoirs are loose within chamber **272**. In such embodiments, actuator **244** may include a porous member having pores too small to permit passage of reservoirs **275** but large enough to permit gas to exit chamber **272**.

Microfluidic devices with various components are described in U.S. provisional application No. 60/553,553 filed Mar. 17, 2004 by Parunak et al., which application is incorporated herein by reference.

While microfluidic device **300** has been described as configured to receive polynucleotides already released from cells, microfluidic devices can be configured to release polynucleotides from cells (e.g., by lysing the cells). For example, referring to FIGS. **4-6**, a microfluidic device **300** includes a sample lysing chamber **302** in which cells are lysed to release polynucleotides therein. Microfluidic device **300** further includes substrate layers L**1**-L**3**, a microfluidic network **304** (only portions of which are seen in FIG. **4**), and liquid reagent reservoirs R**1**-R**4**. Liquid reagent reservoirs R**1**-R**4** hold liquid reagents (e.g., for processing sample material) and are connected to network **304** by reagent ports RP**1**-RP**4**.

Network **304** is substantially defined between layers L**2** and L**3** but extends in part between all three layers L**1**-L**3**. Microfluidic network **304** includes multiple components including channels Ci, valves Vi, double valves V'_i, gates G**1**, mixing gates MGi, vents Hi, gas actuators (e.g., pumps) Pi, a first processing region B**1**, a second processing region B**2**, detection zones Di, air vents AVi, and waste zones Wi. Components of network **304** are typically thermally actuated. As seen in FIG. **7**, a heat source network **312** includes heat sources (e.g., resistive heat sources) having locations that correspond to components of microfluidic network **304**. For example, the locations of heat sources HPi correspond to the locations of actuators Pi, the locations of heat sources HGi correspond to locations of gates G**1** and mixing gates, the

locations of heat sources HVi correspond to the locations of valves Vi and double valves V'i, and the locations of heat sources HD**1** correspond to the locations of processing chambers Di of network **304**. In use, the components of device **300** are disposed in thermal contact with corresponding heat sources of network **312**, which is typically operated using a processor as described above for device **200**. Heat source network **312** can be integral with or separate from device **300** as described for device **200**.

We next discuss components of microfluidic device **300**.

Air vents AVi are components that allow gas (e.g., air) displaced by the movement of liquids within network **304** to be vented so that pressure buildup does not inhibit desired movement of the liquids. For example, air vent AV**2** permits liquid to move along channel C**14** and into channel C**16** by venting gas downstream of the liquid through vent AV**2**.

Valves Vi are components that have a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). The valves Vi can have the same structure as valves of microfluidic device **200**.

As seen in FIGS. **8** and **9**, double valves V'i are also components that have a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). Taking double valve V**11'** of FIGS. **8** and **9** as an example, double valves Vi' include first and second masses **314**, **316** of a TRS (e.g., a eutectic alloy or wax) spaced apart from one another on either side of a channel (e.g., channel C**14**). Typically, the TRS masses **314**, **316** are offset from one another (e.g., by a distance of about 50% of a width of the TRS masses or less). Material moving through the open valve passes between the first and second TRS masses **314**,**316**. Each TRS mass **314**, **316** is associated with a respective chamber **318**, **320**, which typically includes a gas (e.g., air).

The TRS masses **314**, **316** and chambers **318**, **320** of double valve Vi' are in thermal contact with a corresponding heat source HV**11'** of heat source network **312**. Actuating heat source HV**11'** causes TRS masses **314**, **316** to transition to a more mobile second state (e.g., a partially melted state) and increases the pressure of gas within chambers **318**, **320**. The gas pressure drives TRS masses **314**, **316** across channel C**11** and closes valve HV**11'** (FIG. **9**). Typically, masses **314**, **316** at least partially combine to form a mass **322** that obstructs channel C**11**.

Returning to FIG. **6**, gates G**1** are components that have a normally closed state that does not allow material to pass along a channel from a position on one side of the gate to another side of the gate. Gates G**1** can have the same structure as described for gates of device **200**.

As seen in FIGS. **10**A-**10**D, mixing gates MGi are components that allow two volumes of liquid to be combined (e.g., mixed) within network **304**. Mixing gates MGi are discussed further below.

Actuators Pi are components that provide a gas pressure to move material (e.g., sample material and/or reagent material) between one location of network **304** and another location. Actuators Pi can be the same as actuators of device **200**. For example, each actuator Pi includes a chamber with a mass **273** of TEM that can be heated to pressurize gas within the chamber. Each actuator Pi includes a corresponding gate G**1** (e.g., gate G**2** of actuator P**1**) that prevents liquid from entering the chamber of the actuator. The gate is typically actuated (e.g., opened) to allow pressure created in the chamber of the actuator to enter the microfluidic network.

Waste chambers Wi are components that can receive waste (e.g., overflow) liquid resulting from the manipulation (e.g., movement and/or mixing) of liquids within network **304**. Typically, each waste chamber Wi has an associated air vent that allows gas displaced by liquid entering the chamber to be vented.

First processing region B**1** is a component that allows polynucleotides to be concentrated and/or separated from inhibitors of a sample. Processing region B**1** can be configured and operated as processing region **220** of device **200**. In some embodiments, first processing region B**1** includes a retention member (e.g., multiple particles (e.g., microspheres or beads), a porous member, multiple walls) having at least one surface modified with one or more ligands as described for processing region **220**. For example, the ligand can include one or more polyamides (e.g., poly-cationic polyamides such as poly-L-lysine, poly-D-lysine, poly-DL-ornithine). In some embodiments, particles of the retention member are disposed lysing chamber **302** and are moved into processing region B**1** along with sample material.

Second processing region B**2** is a component that allows material (e.g., sample material) to be combined with compounds (e.g., reagents) for determining the presence of one or more polynucleotides. In some embodiments, the compounds include one or more PCR reagents (e.g., primers, control plasmids, and polymerase enzymes). Typically, the compounds are stored within processing region as one or more lyophilized particles (e.g., pellets). The particles generally have a room temperature (e.g., about 20° C.) shelf-life of at least about 6 months (e.g., at least about 12 months). Liquid entering the second processing region B**2** dissolves (e.g., reconstitutes) the lyophilized compounds.

Typically, the lyophilized particle(s) of processing region B**2** have an average volume of about 5 microliters or less (e.g., about 4 microliters or less, about 3 microliters or less, about 2 microliters or less). In some embodiments, the lyophilized particle(s) of processing region B**2** have an average diameter of about 4 mm or less (e.g., about 3 mm or less, about 2 mm or less) In an exemplary embodiment the lyophilized particle(s) have an average volume of about 2 microliters and an average diameter of about 1.35 mm. Lyophilized particles for determining the presence of one or more polynucleotides typically include multiple compounds. In some embodiments, the lyophilized particles include one or more compounds used in a reaction for determining the presence of a polynucleotide and/or for increasing the concentration of the polynucleotide. For example, lypophilized particles can include one or more enzymes for amplifying the polynucleotide as by PCR. We next discuss exemplary lyophilized particles that include exemplary reagents for the amplification of polynucleotides associated with group B streptococcus (GBS) bacteria. In some embodiments, the lyophilized particles include a cryoprotectant, one or more salts, one or more primers (e.g., GBS Primer F and/or GBS Primer R), one or more probes (e.g., GBS Probe—FAM), one or more internal control plasmids, one or more specificity controls (e.g., *Streptococcus pneumoniae* DNA as a control for PCR of GBS), one or more PCR reagents (e.g., dNTPs and/or dUTPs), one or more blocking or bulking agents (e.g., non-specific proteins (e.g., bovine serum albumin (BSA), RNAseA, or gelatin), and a polymerase (e.g., glycerol-free Taq Polymerase). Of course, other components (e.g., other primers and/or specificity controls) can be used for amplification of other polynucleotides.

Cryoprotectants generally help increase the stability of the lypophilized particles and help prevent damage to other compounds of the particles (e.g., by preventing denaturation of

enzymes during preparation and/or storage of the particles). In some embodiments, the cryoprotectant includes one or more sugars (e.g., one or more dissacharides (e.g., trehalose, melizitose, raffinose)) and/or one or more poly-alcohols (e.g., mannitol, sorbitol).

Lyophilized particles can be prepared as desired. Typically, compounds of the lyophilized particles are combined with a solvent (e.g., water) to make a solution, which is then placed (e.g., in discrete aliquots (e.g., drops) such as by pipette) onto a chilled hydrophobic surface (e.g., a diamond film or a polytetrafluorethylene surface). In general, the temperature of the surface is reduced to near the temperature of liquid nitrogen (e.g., about −150° F. or less, about −200° F. or less, about −275° F. or less). The solution freezes as discrete particles. The frozen particles are subjected to a vacuum while still frozen for a pressure and time sufficient to remove the solvent (e.g., by sublimation) from the pellets.

In general, the concentrations of the compounds in the solution from which the particles are made is higher than when reconstituted in the microfluidic device. Typically, the ratio of the solution concentration to the reconstituted concentration is at least about 3 (e.g., at least about 4.5). In some embodiments, the ratio is about 6.

An exemplary solution for preparing lyophilized pellets for use in the amplification of polynucleotides indicative of the presence of GBS can be made by combining a cryoprotecant (e.g., 120 mg of trehalose as dry powder), a buffer solution (e.g., 48 microliters of a solution of 1M Tris at pH 8.4, 2.5M KCl, and 200 mM $MgCl_2$), a first primer (e.g., 1.92 microliters of 500 micromolar GBS Primer F (Invitrogen)), a second primer (e.g., 1.92 microliters of 500 micromolar GBS Primer R (Invitrogen)), a probe (e.g., 1.92 microliters of 250 micromolar GBS Probe—FAM (IDT/Biosearch Technologies)), an control probe (e.g., 1.92 microliters of 250 micromolar Cal Orange 560 (Biosearch Technologies)), a template plasmid (e.g., 0.6 microliters of a solution of 10^5 copies plasmid per microliter), a specificity control (e.g., 1.2 microliters of a solution of 10 nanograms per microliter (e.g., about 5,000,000 copies per microliter) *streptococcus pneumoniae* DNA (ATCC)), PCR reagents (e.g., 4.8 microliters of a 100 millimolar solution of dNTPs (Epicenter) and 4.microliters of a 20 millimolar solution of dUTPs (Epicenter)), a bulking agent (e.g., 24 microliters of a 50 milligram per milliliter solution of BSA (Invitrogen)), a polymerase (e.g., 60 microliters of a 5 U per microliter solution of glycerol-free Taq Polymerase (Invitrogen/Eppendorf)) and a solvent (e.g., water) to make about 400 microliters of solution. About 200 aliquots of about 2 microliters each of this solution are frozen and desolvated as described above to make 200 pellets. When reconstituted, the 200 particles make a PCR reagent solution having a total volume of about 2.4 milliliters.

As seen in FIG. **5**, reagent reservoirs R**1** are configured to hold liquid reagents (e.g., water, buffer solution, hydroxide solution) separated from network **304** until ready for use. Reservoirs R**1** include an enclosure **329** that defines a sealed space **330** for holding liquids. Each space **330** is separated from reagent port RPi and network **304** by a lower wall **33** of enclosure **329**. A portion of enclosure **329** is formed as a piercing member **331** oriented toward the lower wall **333** of each enclosure. When device **300** is to be used, reagent reservoirs R**1** are actuated by depressing piercing member **331** to puncture wall **333**. Piercing member **331** can be depressed by a user (e.g., with a thumb) or by the operating system used to operate device **300**.

When wall **333** is punctured, fluid from the reservoir enters network **333**. For example, as seen in FIGS. **5** and **6**, liquid from reservoir R**2** enters network **304** by port RP**2** and travels along a channel C**2**. Gate G**3** prevents the liquid from passing along channel C**8**. Excess liquid passes along channel C**7** and into waste chamber W**2**. When the trailing edge of liquid from reservoir R**2** passes hydrophobic vent H**2**, pressure created within the reservoir is vented stopping further motion of the liquid. Consequently, network **304** receives an aliquot of liquid reagent having a volume defined by the volume of channel C**2** between a junction J**1** and a junction J**2**. When actuator P**1** is actuated, this aliquot of reagent is moved further within network **304**. Reagent reservoirs R**1**, R**3**, and R**4** are associated with corresponding channels, hydrophobic vents, and actuators.

In the configuration shown, reagent reservoir R**1** typically holds a release liquid (e.g., a hydroxide solution as discussed above for device **200**) for releasing polynucleotides retained within processing region B**1**. Reagent reservoir R**2** typically holds a wash liquid (e.g., a buffer solution as discussed above for device **200**) for removing un-retained compounds (e.g., inhibitors) from processing region B**1** prior to releasing the polynucleotides. Reagent reservoir R**3** typically holds a neutralization buffer (e.g., 25-50 mM Tris-HCl buffer at pH 8.0). Reagent reservoir R**4** typically holds deionized water.

Lysing chamber **302** is divided into a primary lysing chamber **306** and a waste chamber **308**. Material cannot pass from one of chambers **306**, **308** into the other chamber without passing through at least a portion of network **304**. Primary lysing chamber **306** includes a sample input port SP**1** for introducing sample to chamber **306**, a sample output port SP**2** connecting chamber **306** to network **304**, and lyophilized reagent LP that interact with sample material within chamber **306** as discussed below. Input port SP**1** includes a one way valve that permits material (e.g., sample material and gas) to enter chamber **306** but limits (e.g., prevents) material from exiting chamber **308** by port SP**1**. Typically, port SP**1** includes a fitting (e.g., a Luer fitting) configured to mate with a sample input device (e.g., a syringe) to form a gas-tight seal. Primary chamber **306** typically has a volume of about 5 milliliters or less (e.g., about 4 milliliters or less). Prior to use, primary chamber **306** is typically filled with a gas (e.g., air).

Waste chamber **308** includes a waste portion W**6** by which liquid can enter chamber **308** from network **304** and a vent **310** by which gas displaced by liquid entering chamber **308** can exit.

Lyophilized reagent particles LP of lysing chamber **302** include one or more compounds (e.g., reagents) configured to release polynucleotides from cells (e.g., by lysing the cells). For example, particles LP can include one or more enzymes configured to reduce (e.g., denature) proteins (e.g., proteinases, proteases (e.g., pronase), trypsin, proteinase K, phage lytic enzymes (e.g., PlyGBS)), lysozymes (e.g., a modified lysozyme such as ReadyLyse), cell specific enzymes (e.g., mutanolysin for lysing group B streptococci)).

In some embodiments, articles LP typically alternatively or additionally include components for retaining polynucleotides as compared to inhibitors. For example, particles LP can include multiple particles **218** surface modified with ligands as discussed above for device **200**. Particles LP can include enzymes that reduce polynucleotides that might compete with a polynucleotide to be determined for binding sites on the surface modified particles. For example, to reduce RNA that might compete with DNA to be determined, particles LP may include an enzyme such as an RNAase (e.g., RNAseA ISC BioExpress (Amresco)).

In an exemplary embodiment, particles LP cells include a cryoprotecant, particles modified with ligands configured to retain polynucleotides as compared to inhibitors, and one or more enzymes.

Typically, particles LP have an average volume of about 35 microliters or less (e.g., about 27.5 microliters or less, about 25 microliters or less, about 20 microliters or less). In some embodiments, the particles LP have an average diameter of about 8 mm or less (e.g., about 5 mm or less, about 4 mm or less) In an exemplary embodiment the lyophilized particle(s) have an average volume of about 20 microliters and an average diameter of about 3.5 mm.

Particles LP can be prepared as desired. Typically, the particles are prepared using a cryoprotectant and chilled hydrophobic surface as described above. For example, a solution for preparing particles LP can be prepared by combining a cryoprotectant (e.g., 6 grams of trehalose), a plurality of particles modified with ligands (e.g., about 2 milliliters of a suspension of carboxylate modified particles with poly-D-lysine ligands), a protease (e.g., 400 milligrams of pronase), an RNAase (e.g., 30 milligrams of RNAseA (activity of 120 U per milligram), an enzyme that digests peptidoglycan (e.g., ReadyLyse (e.g., 160 microliters of a 30000 U per microliter solution of ReadyLyse)), a cell specific enzyme (e.g., mutanolysin (e.g., 200 microliters of a 50 U per microliter solution of mutanolysin), and a solvent (e.g., water) to make about 20 milliliters. About 1000 aliquots of about 20 microliters each of this solution are frozen and desolvated as described above to make 1000 pellets. When reconstituted, the pellets are typically used to make a total of about 200 milliliters of solution.

In use, device **300** can be operated as follows. Valves Vi and Vi' of network **304** are configured in the open state. Gates G**1** and mixing gates MGi of network **304** are configured in the closed state. Reagent ports R**1**-R**4** are depressed to introduce liquid reagents into network **304** as discussed above. A sample is introduced to lysing chamber **302** via port SP**1** and combined with lyophilized particles LP within primary lysing chamber **306**. Typically, the sample includes a combination of particles (e.g., cells) and a buffer solution. For example, an exemplary sample includes about 2 parts whole blood to 3 about parts buffer solution (e.g., a solution of 20 mM Tris at pH 8.0, 1 mM EDTA, and 1% SDS). Another exemplary sample includes group B streptococci and a buffer solution (e.g., a solution of 20 mM Tris at pH 8.0, 1 mM EDTA, and 1% Triton X-100).

In general, the volume of sample introduced is smaller than the total volume of primary lysing chamber **306**. For example, the volume of sample may be about 50% or less (e.g., about 35% or less, about 30% or less) of the total volume of chamber **306**. A typical sample has a volume of about 3 milliliters or less (e.g., about 1.5 milliliters or less). A volume of gas (e.g., air) is generally introduced to primary chamber **306** along with the sample. Typically, the volume of gas introduced is about 50% or less (e.g., about 35% or less, about 30% or less) of the total volume of chamber **306**. The volume of sample and gas combine to pressurize the gas already present within chamber **306**. Valve **307** of port SP**1** prevents gas from exiting chamber **306**. Because gates G**3**, G**4**, G**8**, and G**10** are in the closed state, the pressurized sample is prevented from entering network **304** via port SP**2**.

The sample dissolves particles LP in chamber **306**. Reconstituted lysing reagents (e.g., ReadyLyse, mutanolysin) begin to lyse cells of the sample releasing polynucleotides. Other reagents (e.g., protease enzymes such as pronase) begin to reduce or denature inhibitors (e.g., proteins) within the sample. Polynucleotides from the sample begin to associate with (e.g., bind to) ligands of particles **218** released from particles LP. Typically, the sample within chamber **306** is heated (e.g., to at least about 50° C., to at least about 60° C.) for a period of time (e.g., for about 15 minutes or less, about 10 minutes or less, about 7 minutes or less) while lysing occurs. In some embodiments, optical energy is used at least in part to heat contents of lysing chamber **306**. For example, the operating system used to operate device **300** can include a lamp (e.g., a lamp primarily emitting light in the infrared) disposed in thermal and optical contact with chamber **306**. Chamber **306** includes a temperature sensor TS used to monitor the temperature of the sample within chamber **306**. The lamp output is increased or decreased based on the temperature determined with sensor TS.

Continuing with the operation of device **300**, G**2** is actuated (e.g., opened) providing a path between port SP**2** of primary lysing chamber **306** and port W**6** of lysing waste chamber **308**. The path extends along channel C**9**, channel C**8**, through processing region B**1**, and channel C**11**. Pressure within chamber **306** drives the lysed sample material (containing lysate, polynucleotides bound to particles **218**, and other sample components) along the pathway. Particles **218** (with polynucleotides) are retained within processing region B**1** (e.g., by a filter) while the liquid and other components of the sample flow into waste chamber **308**. After a period of time (e.g., between about 2 and about 5 minutes), the pressure in lysing chamber **306** is vented by opening gate G**1** to create a second pathway between ports SP**2** and W**6**. Double valves V**1'** and V**8'** are closed to isolate lysing chamber **302** from network **304**.

Operation of device **300** continues by actuating pump P**1** and opening gates G**2**,G**3** and G**9**. Pump P**1** drives wash liquid in channel C**2** downstream of junction J**1** through processing region B**1** and into waste chamber W**5**. The wash liquid removes inhibitors and other compounds not retained by particles **218** from processing region B**1**. When the trailing edge of the wash liquid (e.g., the upstream interface) passes hydrophobic vent H**14**, the pressure from actuator P**1** vents from network **304**, stopping further motion of the liquid. Double valves V**2'** and V**9'** are closed.

Operation continues by actuating pump P**2** and opening gates G**6**, G**4** and G**8** to move release liquid from reagent reservoir R**1** into processing region B**1** and into contact with particles **218**. Air vent AV**1** vents pressure ahead of the moving release liquid. Hydrophobic vent H**6** vents pressure behind the trailing edge of the release liquid stopping further motion of the release liquid. Double valves V**6'** and V**10'** are closed.

Operation continues by heating processing region B**1** (e.g., by heating particles **218**) to release the polynucleotides from particles **218**. The particles can be heated as described above for device **200**. Typically, the release liquid includes about 15 mM hydroxide (e.g., NaOH solution) and the particles are heated to about 70° C. for about 2 minutes to release the polynucleotides from the particles **218**.

Operation continues by actuating pump P**3** and opening gates G**5** and G**10** to move release liquid from process region B**1** downstream. Air vent AV**2** vents gas pressure downstream of the release liquid allowing the liquid to move into channel C**16**. Hydrophobic vent H**8** vents pressure from upstream of the release liquid stopping further movement. Double valve V**11'** and valve V**14** are closed.

Referring to FIGS. **10**A-**10**D, mixing gate MG**11** is used to mix a portion of release liquid including polynucleotides released from particles **218** and neutralization buffer from reagent reservoir R**3**. FIG. **10**A shows the mixing gate MG**11** region prior to depressing reagent reservoir R**3** to introduce the neutralization buffer into network **304**. FIG. **10**B shows the mixing gate MG**11** region, after the neutralization buffer has been introduced into channels C**13** and C**12**. Double valve V**13'** is closed to isolate network **304** from reagent reservoir

R3. Double valve V12' is closed to isolate network 304 from waste chamber W3. The neutralization buffer contacts one side of a mass 324 of TRS of gate MG11.

FIG. 10*c* shows the mixing gate MG11 region after release liquid has been moved into channel C16. The dimensions of microfluidic network 304 (e.g., the channel dimensions and the position of hydrophobic vent H8) are configured so that the portion of release liquid positioned between junctions J3 and J4 of channels C16 and C14 corresponds approximately to the volume of liquid in contact with particles 218 during the release step. In some embodiments, the volume of liquid positioned between junctions J3 and J4 is less than about 5 microliters (e.g., about 4 microliters or less, about 2.5 microliters or less). In an exemplary embodiment the volume of release liquid between junctions J3 and J4 is about 1.75 microliters. Typically, the liquid between junctions J3 and J4 includes at least about 50% of polynucleotides (at least about 75%, at least about 85%, at least about 90%) of the polynucleotides present in the sample that entered processing region B1. Valve V14 is closed to isolate network 304 from air vent AV2.

Before actuating mixing gate MG11, the release liquid at junction J4 and the neutralization buffer at a junction J6 between channels C13 and C12 are separated only be mass 324 of TRS (e.g., the liquids are not spaced apart by a volume of gas). To combine the release liquid and neutralization buffer, pump P4 and gates G12, G13, and MG11 are actuated. Pump P4 drives the volume of neutralization liquid between junctions J5 and J6 and the volume of release liquid between junctions J4 and J3 into mixing channel C15 (FIG. 10D). Mass 324 of TRS typically disperses and/or melts allowing the two liquids to combine. The combined liquids include a downstream interface 335 (formed by junction J3) and an upstream interface (formed by junction J5). The presence of these interfaces allows more efficient mixing (e.g., recirculation of the combined liquid) than if the interfaces were not present. As seen in FIG. 10D, mixing typically begins near the interface between the two liquids. Mixing channel C15 is typically at least about as long (e.g., at least about twice as long) as a total length of the combined liquids within the channel.

The volume of neutralization buffer combined with the release liquid is determined by the channel dimensions between junction J5 and J6. Typically, the volume of combined neutralization liquid is about the same as the volume of combined release liquid. In some embodiments, the volume of liquid positioned between junctions J5 and J6 is less than about 5 microliters (e.g., about 4 microliters or less, about 2.5 microliters or less). In an exemplary embodiment the volume of release liquid between junctions J5 and J6 is about 2.25 microliters (e.g., the total volume of release liquid and neutralization buffer is about 4 microliters).

Returning to FIG. 6, the combined release liquid and neutralization buffer move along mixing channel C15 and into channel C32 (vented downstream by air vent AV8). Motion continues until the upstream interface of the combined liquids passes hydrophobic vent H11, which vents pressure from actuator P4 stopping further motion of the combined liquids.

Continuing with operation of device 300, actuator P5 and gates G14, G15 and G17 are actuated to dissolve the lyophilized PCR particle present in second processing region B2 in water from reagent reservoir R4. Hydrophobic vent H10 vents pressure from actuator P5 upstream of the water stopping further motion. Dissolution typically occurs in about 2 minutes or less (e.g., in about 1 minute or less). to dissolve PCR-reagent pellet. Valve V17 is closed.

Continuing with operation of device 300, actuator P6 and gate G16 are actuated to drive the dissolved compounds of the lyophilized particle from processing region B2 into channel C31, where the dissolved reagents mix to form a homogenous dissolved lyophilized particle solution. Actuator P6 moves the solution into channels C35 and C33 (vented downstream by air vent AV5). Hydrophobic vent H9 vents pressure generated by actuator P6 upstream of the solution stopping further motion. Valves V18, V19, V20', and V22' are closed.

Continuing with operation of device 300, actuator P7 and gates G18, MG20 and G22 are actuated to combine (e.g., mix) a portion of neutralized release liquid in channel 32 between gate MG20 and gate G22 and a portion of the dissolved lyophilized particle solution in channel C35 between gate G18 and MG20. The combined liquids travel long a mixing channel C37 and into detection region D2. An air vent AV3 vents gas pressure downstream of the combined liquids. When the upstream interface of the combined liquids passes hydrophobic vent H13, the pressure from actuator P7 is vented and the combined liquids are positioned within detection region D2.

Actuator P8 and gates MG2, G23, and G19 are actuated to combine a portion of water from reagent reservoir R4 between MG2 and gate G23 with a second portion of the dissolved lyophilized particle solution in channel C33 between gate G19 and MG2. The combined liquids travel long a mixing channel C41 and into detection region D1. An air vent AV4 vents gas pressure downstream of the combined liquids. When the upstream interface of the combined liquids passes hydrophobic vent H12, the pressure from actuator P8 is vented and the combined liquids are positioned within detection region D1.

Continuing with operation of device 300, double valves V26' and V27' are closed to isolate detection region D1 from network 304 and double valves V24' and V25' are closed to isolate detection region D2 from network 304. The contents of each detection region (neutralized release liquid with sample polynucleotides in detection region D2 with PCR reagents from dissolved lyophilized particle solution and deionized water with PCR reagents from dissolved lyophilized particle solution in detection region D1) are subjecting to heating and cooling steps to amplify polynucleotides (if present in detection region D2). The double valves of each detection region prevent evaporation of the detection region contents during heating. The amplified polynucleotides are typically detected using fluorescence detection.

Referring to FIG. 11, a device 700 is configured to process a polynucleotide-containing sample, such as to prepare the sample for amplification of the polynucleotides. Device 700 includes a sample reservoir 704, a reagent reservoir 706, a gas pressure generator 708, a closure (e.g., a cap 710), and a processing region 702 including a retention member 704 having a plurality of particles (e.g. carboxylate beads 705 surface-modified with a ligand, e.g., poly-L-lysine and/or poly-D-lysine). Retention member 705 and beads 705 may share any or all properties of retention member 216 and surface-modified particles 218. Device 700 also includes an opening 716 and a valve, e.g., a thermally actuated valve 714 for opening and closing opening 716.

In use, a polynucleotide-containing sample is added to sample reservoir 704. Typical sample amounts range from about 100 μL to about 2 mL, although greater or smaller amounts may be used.

Reagent reservoir 706 may be provided to users of device 700 with pre-loaded reagent. Alternatively, device 700 may be configured so that users add reagent to device 700. In any

event, the reagents may include, e.g., NaOH solutions and/or buffer solutions such as any of such solutions discussed herein.

Once sample and, if necessary, reagent have been added to device **700**, cap **710** is closed to prevent evaporation of sample and reagent materials.

Referring also to FIG. **12**, an operator **718** is configured to operate device **700**. Operator **718** includes a first heat source **720** and a second heat source **722**. First heat source **720** heats sample present within sample reservoir **704**, such as to lyse cells of the polynucleotide-containing sample to prepare free polynucleotides.

Device **700** may also include an enzyme reservoir **712** comprising an enzyme, e.g., a protease such as pronase, configured to cleave peptide bonds of polypeptides present in the polynucleotide-containing sample. Enzyme reservoir **712** may be provided to users of device **700** with pre-loaded enzyme. Alternatively, device **700** may be configured so that users add enzyme to device **700**.

Device **700** may be used to reduce the amount of inhibitors present relative to the amount of polynucleotides to be determined. Thus, the sample is eluted through processing region **702** to contact constituents of the sample with beads **705**. Beads **705** retain polynucleotides of the sample as compared to inhibitors as described elsewhere herein. With valve **714** in the open state, sample constituents not retained in processing region **702** exit device **700** via the opening.

Once the polynucleotide-containing sample has eluted through processing region **702**, an amount of reagent, e.g., a wash solution, e.g., a buffer such as Tris-EDTA pH 8.0 with 1% Triton X 100 is eluted through processing region **702**. The wash solution is generally stored in reagent reservoir **706**, which may include a valve configured to release an amount of wash solution. The wash solution elutes remaining polynucleotide-containing sample and inhibitors without eluting retained polynucleotides.

Once inhibitors have been separated from retained polynucleotides, the polynucleotides are released from beads **705**. In some embodiments, polynucleotides are released by contacting the beads **705** with a release solution, e.g., a NaOH solution or buffer solution having a pH different from that of the wash solution. Alternatively, or in combination, beads **705** with retained polynucleotides are heated, such as by using second heat source **722** of operator **718**. When heat is used to release the polynucleotides, the release solution may be identical with the wash solution.

Gas pressure generator **708** may be used to expel an amount of release solution with released polynucleotides from device **700**. Gas pressure generator and/or operator **718** may include a heat source to heat gas present within generator **708**. The heated gas expands and provides the gas pressure to expel sample. In some embodiments, and whether or not thermally generated gas pressure is used, gas pressure generator **708** is configured to expel a predetermined volume of material. Typically, the amount of expelled solution is less than about 500 μL, less than about 250 μL, less than about 100 μL, less than about 50 μL, e.g., less than about 25 μL.

EXAMPLES

The following Examples are illustrative and not intended to be limiting.

Preparing Retention Member

Carboxylate surface magnetic beads (Sera-Mag Magnetic Carboxylate modified, Part #3008050250, Seradyn) at a concentration of about 10^{11} mL^{-1} were activated for 30 minutes using N-hydroxylsuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) in a pH 6.1 500 mM 2-(N-Morpholinio)-ethanesulfonic acid (MES) buffer solution. Activated beads were incubated with 3000 Da or 300,000 Da average molecular weight poly-L-lysine (PLL). After 2 washes to remove unbound PLL, beads were ready for use.

Microfluidic Device

Referring to FIGS. **13** and **14**, a microfluidic device **300** was fabricated to demonstrate separation of polynucleotides from inhibitors. Device **300** comprises first and second substrate portions **302'**, **304'**, which respectively comprise first and second layers **302***a*', **302***b*' and **304***a*', **304***b*'. First and second layers **302***a*', **302***b*' define a channel **306'** comprising an inlet **310'** and an outlet **312'**. First and second layers **304***a*', **304***b*' define a channel **308'** comprising an inlet **314'** and an outlet **316'**. First and second substrate portions **302'**, **304'** were mated using adhesive **324'** so that outlet **312'** communicated with inlet **314'** with a filter **318'** positioned therebetween. A portion of outlet **312'** was filed with the activated beads prepared above to provide a processing region **320'** comprising a retention member (the beads). A pipette **322'** (FIG. **14**) secured by adhesive **326'** facilitated sample introduction.

In use, sample introduced via inlet **310'** passed along channel and through processing region **320'**. Excess sample material passed along channel **308'** and exited device **300'** via outlet **316'**. Polynucleotides were preferentially retained by the beads as compared to inhibitors. Once sample had been introduced, additional liquids, e.g., a wash liquid and/or a liquid for use in releasing the retained polynucleotides were introduced via inlet **326'**.

Retention of DNA

Retention of polynucleotides by the poly-L-lysine modified beads of device **300'** was demonstrated by preparing respective devices comprising processing regions having a volume of about 1 mL including about 1000 beads. The beads were modified with poly-L-lysine of between about 15,000 and 30,000 Da. Each processing region was filled with a liquid comprising herring sperm DNA (about 20 uL of sample with a concentration of about 20 mg/mL) thereby placing the beads and liquid in contact. After the liquid and beads had been in contact for 10 minutes, the liquid was removed from each processing region and subjected to quantitative real-time PCR to determine the amount of herring sperm DNA present in the liquid.

Two controls were performed. First, an otherwise identical processing region was packed with unmodified beads, i.e., beads that were identical with the poly-L-lysine beads except for the activation and poly-L-lysine incubation steps. The liquid comprising herring sperm DNA was contacted with these beads, allowed to stand for 10 minutes, removed, and subjected to quantitative real-time PCR. Second, the liquid comprising the herring sperm DNA ("the unprocessed liquid") was subjected to quantitative real-time PCR.

Referring to FIG. **15**, the first and second controls exhibited essentially identical responses indicating the presence of herring sperm DNA in the liquid contacted with the unmodified beads and in the unprocessed liquid. The liquid that had contacted the 3,000 poly-L-lysine beads exhibited a lower response indicating that the modified beads had retained substantially all of the herring sperm DNA. The PCR response of the liquid that had contacted the 300,000 Da poly-L-lysine beads exhibited an amplification response that was at least about 50% greater than for the 3,000 Da beads indicating that the lower molecular weight surface modification was more efficient at retaining the herring sperm DNA.

Releasing DNA from Poly-L-Lysine Modified Beads

Devices having processing regions were packed with 3,000 Da poly-L-lysine modified beads. Liquid comprising polynucleotides obtained from group B streptococci (GBS) was contacted with the beads and incubated for 10 minutes as above for the herring sperm DNA. This liquid had been obtained by subjecting about 10,000 GBS bacteria in 10 μl of 20 mM Tris pH 8, 1 mM EDTA, 1% Triton X-100 buffer to thermal lysing at 97° C. for 3 min.

After 10 minutes, the liquid in contact with the beads was removed by flowing about 10 μl of wash solution (Tris-EDTA pH 8.0 with 1% Triton X 100) through the processing region. Subsequently, about 1 μl of 5 mM NaOH solution was added to the processing region. This process left the packed processing region filled with the NaOH solution in contact with the beads. The solution in contact with the beads was heated to 95° C. After 5 minutes of heating at 95° C., the solution in contact with the beads was removed by eluting the processing region with a volume of solution equal to three times the void volume of the processing region.

Referring to FIG. **16**, five aliquots of solution were subjected to quantitative real-time PCR amplification. Aliquots E**1**, E**2**, and E**3** each contained about 1 μl of liquid. Aliquot L was corresponds to liquid of the original sample that had passed through the processing region. Aliquot W was liquid obtained from wash solution without heating. Aliquot E**1** corresponds to the dead volume of device **300**, about equal to the volume of channel **308**. Thus, liquid of aliquot E**1** was present in channel **308** and not in contact with the beads during heating. This liquid had passed through the processing region prior to heating. Aliquot E**2** comprises liquid that was present within the processing region and in contact with the beads during heating. Aliquot E**3** comprises liquid used to remove aliquot E**2** from the processing region.

As seen in FIG. **16**, more than 65% of the GBS DNA present in the initial sample was retained by and released from the beads (Aliquot E**2**). Aliquot E**2** also demonstrates the release of more than 80% of the DNA that had been retained by the beads. Less than about 18% of the GBS DNA passed through the processing region without being captured. The wash solution without heating comprised less than 5% of the GBS DNA (Aliquot W).

Separation of Polynucleotides and Inhibitors

Buccal cells from the lining of the cheeks provide a source of human genetic material (DNA) that may be used for single nucleotide polymorphism (SNP) detection. A sample comprising buccal cells was subjected to thermal lysing to release DNA from within the cells. Device **300** was used to separate the DNA from concomitant inhibitors as described above. A cleaned-up sample corresponding to aliquot E**2** of FIG. **16** was subjected to polymerase chain reaction. A control or crude sample as obtained from the thermal lysing was also amplified.

Referring to FIG. **17**, the cleaned-up sample exhibited substantially higher PCR response in fewer cycles than did the control sample. For example, the clean-up sample exceeded a response of 20 within 32 cycles whereas the control sample required about 45 cycles to achieve the sample response.

Blood acts as a sample matrix in variety of diagnostic tests including detection of infectious disease agents, cancer markers and other genetic markers. Hemoglobin present in blood samples is a documented potent inhibitor of PCR. Two 5 ml blood samples were lysed in 20 mM Tris pH 8, 1 mM EDTA, 1% SDS buffer and introduced to respective devices **300**, which were operated as described above to prepare two clean-up samples. A third 5 ml blood sample was lysed and prepared using a commercial DNA extraction method Puregene, Gentra Systems, MN. The respective cleaned-up samples and sample subjected to the commercial extraction method were used for a Allelic discrimination analysis (CYP2D6*4 reagents, Applied Biosystems, CA). Each sample contained an amount of DNA corresponding to about 1 ml of blood.

Referring to FIG. **18**, the cleaned-up and commercially extracted samples exhibited similar PCR response demonstrating that the processing region of device **300'** efficiently removed inhibitors from the blood samples.

Protease Resistant Retention Member

The preparation of polynucleotide samples for further processing often includes subjecting the samples to protease treatment in which a protease cleaves peptide bonds of proteins in the sample. An exemplary protease is pronase, a mixture of endo- and exo-proteases. Pronase cleaves most peptide bonds. Certain ligands, such as poly-L-lysine are susceptible to rupture by pronase and other proteases. Thus, if samples are generally not subjected to protease treatment in the presence of the retention member if the ligands bound thereto are susceptible to the proteases.

Poly-D-lysine, the dextro enantiomer of poly-lysine resists cleavage by pronase and other proteases. The ability of a retention member comprising bound poly-D-lysine to retain DNA even when subjected to a protease treatment was studied.

Eight (8) samples were prepared. A first group of 4 samples contained 1000 GBS cells in 10 μl buffer. A second group of 4 samples contained 100 GBS cells in 10 μl buffer. Each of the 8 samples was heated to 97° C. for 3 min to lyse the GBS cells. Four (4) sample sets were created from the heated samples. Each sample set contained 1 sample from each of the first and second groups. The samples of each sample sets were treated as follows.

Referring to FIG. **19***a*, the samples of sample set 1 were subjected to pronase incubation to prepare respective protein cleaved samples, which were then heated to inactivate the proteases. The protein-cleaved, heated samples were contacted with respective retention members each comprising a set of poly-L-lysine modified beads. After 5 minutes, the respective sets of beads were washed with 5 microliters of a 5 mM NaOH solution to separate inhibitors and products of protein cleavage from the bound DNA. The respective sets of beads were each contacted with a second aliquot of NaOH solution and heated to 80 (eighty) ° C. for 2 minutes to release the DNA. The solutions with released DNA were neutralized with an equal volume of buffer. The neutralized solutions were analyzed to determine the efficiency of DNA recovery. The results were averaged and shown in FIG. **19***b*.

The samples of sample set 2 were subjected to pronase incubation to prepare respective protein cleaved samples, which were then heated to inactivate the proteases. The protein-cleaved, heated samples were contacted with respective retention members each comprising a set of poly-D-lysine modified beads. After 5 minutes, the respective sets of beads were washed with 5 microliters of a 5 mM NaOH solution to separate inhibitors and products of protein cleavage from the bound DNA. The respective sets of beads were each contacted with a second aliquot of NaOH solution and heated to 80 (eighty) ° C. for 2 minutes to release the DNA. The solutions with released DNA were neutralized with an equal volume of buffer. The neutralized solutions were analyzed to determine the efficiency of DNA recovery. The results were averaged and shown in FIG. **19***b*.

The samples of sample set 3 were subjected to pronase incubation to prepare respective protein cleaved samples. The proteases were not deactivated either thermally or chemi-

cally. The protein-cleaved samples were contacted with respective retention members each comprising a set of poly-L-lysine modified beads. After 5 minutes, the respective sets of beads were washed with 5 microliters of a 5 mM NaOH solution to separate inhibitors and products of protein cleavage from the bound DNA. The respective sets of beads were each contacted with a second aliquot of NaOH solution and heated to 80 (eighty) ° C. for 2 minutes to release the DNA. The solutions with released polynucleotides were each neutralized with an equal volume of buffer. The neutralized solutions were analyzed to determine the efficiency of DNA recovery. The results were averaged and shown in FIG. **19***b*.

The samples of sample set 4 were subjected to pronase incubation to prepare respective protein cleaved samples. The proteases were not deactivated either thermally or chemically. The protein-cleaved samples were contacted with respective retention members each comprising a set of poly-D-lysine modified beads. After 5 minutes, the respective sets of beads were washed with 5 microliters of a 5 mM NaOH solution to separate inhibitors and products of protein cleavage from the bound DNA. The respective sets of beads were each contacted with a second aliquot of NaOH solution and heated to 80 (eighty) ° C. for 2 minutes to release the DNA. The solutions with released polynucleotides were each neutralized with an equal volume of buffer. The neutralized solutions were analyzed to determine the efficiency of DNA recovery. The results were averaged and shown in FIG. **19***b*.

As seen in FIG. **19***b*, an average of more than 80% of DNA from the GBS cells was recovered using sample set 4 in which the samples were contacted with poly-D-lysine modified beads and subjected to pronase incubation in the presence of the beads without protease inactivation. The recovery efficiency for sample set 4 is more than twice as high as for any of the other samples. Specifically, the recovery efficiencies for sample sets 1, 2, 3, and 4, were 29%, 32%, 14%, and 81.5%, respectively. The efficiencies demonstrate that high recovery efficiencies can be obtained for samples subjected to protease incubation in the presence of a retention member that retains DNA.

Other embodiments are within the claims.

What is claimed is:

1. A microfluidic device, comprising:

a processing region having a vertical central axis therethrough, the processing region comprised of an inlet, a retention member, a filter, and an outlet;

said retention member being configured to preferentially retain one or more polynucleotides in a sample as compared to polymerase chain reaction inhibitors in the sample, wherein said retention member comprises a plurality of polynucleotide binding particles, and said plurality of binding particles having surfaces that comprise a poly-cationic polyamide bound thereto, further wherein the retention member is configured to retain polynucleotides from a sample preferentially to inhibitors in the sample as the sample passes through the retention member from the processing region inlet to the processing region outlet;

wherein the vertical central axis of the processing region is perpendicular to a horizontal plane of the microfluidic device;

wherein the processing region outlet is disposed along the vertical central axis above the processing region inlet;

wherein the filter is configured to prevent the plurality of binding particles from passing downstream of the processing region;

a device inlet in communication with, and upstream of, the processing region;

a device outlet in communication with, and downstream of, the processing region; and

a detection region in fluid communication with and downstream of the retention member.

2. The microfluidic device of claim **1**, wherein the plurality of particles have a volume less than 5 microliters.

3. The microfluidic device of claim **1**, wherein the sample has a volume from 0.5 microliters to 3 milliliters.

4. The microfluidic device of claim **1**, wherein the poly-cationic polyamide comprises at least one of poly-DL-ornithine, PEI, poly-L-lysine and poly-D-lysine.

5. The microfluidic device of claim **1**, wherein the filter is disposed between the retention member and the processing region outlet.

6. The microfluidic device of claim **1**, further comprising at least one channel having at least one sub-millimeter cross-sectional dimension.

7. The microfluidic device of claim **1**, further comprising a valve, wherein the valve includes a mass of thermally responsive substance that is relatively immobile at a first temperature and more mobile at a second temperature.

8. The microfluidic device of claim **1**, further comprising a gate, wherein the gate includes a mass of thermally responsive substance which is relatively immobile at a first temperature and which, upon heating to a second temperature, changes state.

9. The microfluidic device of claim **1**, further comprising a fluid reservoir configured to hold a liquid.

10. The microfluidic device of claim **9**, wherein the reservoir comprises a liquid having a pH of at least about 10.

11. The microfluidic device of claim **9**, wherein the device is configured to contact the retention member with the liquid.

12. The microfluidic device of claim **1**, further comprising an actuator, wherein the actuator comprises a chamber having a mass of thermally expansive material therein, and a gas.

13. The microfluidic device of claim **1**, further comprising a hydrophobic vent, wherein the vent comprises a layer of porous hydrophobic material.

14. The microfluidic device of claim **1**, having three layers of polymeric material.

15. The microfluidic device of claim **1**, thermally associated with an array of heat sources.

16. The microfluidic device of claim **15**, wherein at least one of the heat sources is configured to heat an aqueous liquid in contact with the retention member to at least about 65° C.

17. The microfluidic device of claim **16**, wherein the liquid is held in a pouch, isolated from the reservoir by a generally impermeable membrane.

18. The microfluidic device of claim **17**, wherein the pouch additionally comprises a piercing member configured to puncture the impermeable membrane.

19. The microfluidic device of claim **1**, further comprising one or more PCR reagents stored as one or more lyophilized particles.

20. The microfluidic device of claim **19**, wherein the lyophilized particles have an average volume of about 5 microliters or less.

21. The microfluidic device of claim **1**, further comprising one or more lysing reagents stored as one or more lyophilized particles.

22. The microfluidic device of claim **21**, wherein the lyophilized particles have an average volume of about 35 microliters or less.

23. The microfluidic device of claim **1**, additionally comprising a lysing chamber and a waste chamber.

24. The microfluidic device of claim **1**, wherein the particles are made of a polymeric material selected from the

group consisting of: polystyrene, latex polymers, polyacrylamide, and polyethylene oxide.

25. The microfluidic device of claim **24**, wherein the polymeric material is modified to include one or more carboxylic groups and/or one or more amino groups covalently bound to the polymeric material, wherein the groups provide an attachment point for one or more ligands of the poly-cationic polyamide.

26. The microfluidic device of claim **1**, wherein the particles have an average diameter of between about 4 microns and about 20 microns.

27. The microfluidic device of claim **1**, wherein the particles are present in a density of about 10^8 particles per milliliter.

28. The microfluidic device of claim **1**, wherein at least some of the particles are magnetic.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 8,470,586 B2
APPLICATION NO. : 11/579353
DATED : June 25, 2013
INVENTOR(S) : Betty Wu et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page (Item 87) PCT Pub. No., Line 1, change "WO2005/018620" to --WO2005/108620--.

Title Page 7, References Cited, Column 1, Line 21 (Item 56), under Other Publications, change "Microfludic" to --Microfluidic--.

Title Page 7, References Cited, Column 1, Line 31 (Item 56), under Other Publications, change "Micochannel"," to --Microchannel",--.

Title Page 7, References Cited, Column 2, Line 4 (Item 56), under Other Publications, change "Concentraction" to --Concentration--.

Title Page 7, References Cited, Column 2, Line 17 (Item 56), under Other Publications, change "fro" to --for--.

In the Specification

Column 2, Line 58, change "mucousol" to --mucosal--.

Column 4, Line 48, change "lamp)." to --lamp)).--.

Column 5, Line 1, change "FIG. 3." to --FIG. 3--.

Column 5, Line 61, change "mucousal" to --mucosal--.

Column 7, Line 1, change "0.2 mm" to --0.2 mm2--.

Column 16, Line 47, change "lypophilized" to --lyophilized--.

Signed and Sealed this
Sixth Day of May, 2014

Michelle K. Lee

Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office

Column 16, Line 66, change “lypophilized” to --lyophilized--.

Column 17, Line 3, change “dissacharides” to --disaccharides--.

Column 17, Line 4, change “melizitose” to --melezitose--.

Column 17, Line 26, change “cryoprotecant” to --cryoprotectant--.

Column 17, Line 41, change “4.microliters” to --4 microliters--.

Column 18, Line 65, change “cryoprotecant,” to --cryoprotectant,--.

Column 19, Line 23, change “milliters.” to --milliliters.--.

Column 20, Line 28, change “G2,G3” to --G2, G3--.

Column 24, Line 37 , change “1 mL” to --1 µL--.

Column 24, Line 40, change “20 uL” to --20 µL--.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 8,470,586 B2
APPLICATION NO. : 11/579353
DATED : June 25, 2013
INVENTOR(S) : Wu et al.

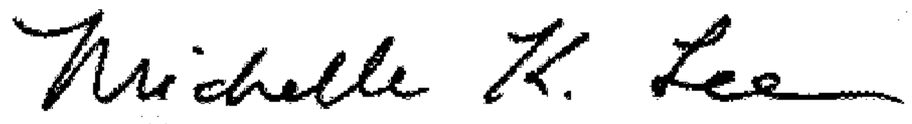
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

The first or sole Notice should read --

Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 740 days.

Signed and Sealed this
Eighth Day of September, 2015



Michelle K. Lee
Director of the United States Patent and Trademark Office